REMARKS

This Amendment is filed in response to the Office Action dated February 27, 2009.

Rejection Under 35 U.S.C. § 102(b):

Claim 1 was rejected under 35 U.S.C. § 102(b) as being anticipated by Hartl et al. (U.S. Patent No. 4,225,557) in view of Roe et al. (U.S. Patent No. 6,060,039). Hartl et al. recites: "A packaged diagnostic test strip for determining occult blood in a stool sample, ...**said back sheet having at least one flap-covered aperture therein in that region thereof corresponding to the location of said aperture in said front sheet, whereby when the flap is opened said test strip is exposed thereunder for application of peroxide solution thereto to develop the reagent present in said test strip; and a closure flap at least partially extending over said front sheet and having closure means thereon for covering said aperture in said front sheet when in closed position**" (emphasis added) (Claim 1, Column 4, Lines 36-37 and Lines 47-56). Therefore, in order to receive the peroxide solution, the closure flap needs to be open. If the foldable sheet is permeable, the closure flap would not provide any function. Therefore, a person of ordinary skill in the art would assume that the closure flap is liquid impermeable by a reading of Hartl et al. In fact, Hartl et al. makes this point very clear: "This part of the test can be performed by the patient himself. After closure, i.e. by insertion of the tab on the cover flap into the slit, the test slide goes to the doctor. **The latter opens the flaps on the back sheet and then applies the developer e.g. (peroxide solution) to the portions of the intermediate sheet, impregnated with the test reagent, which are so exposed and observes the results.** When guaicum resin is employed as an indicator in a test for occult blood in the stool, a blue to blue-green

coloration indicates a positive result" (emphasis added) (Column 1, Lines 58-68). Therefore, the diagnostic test strip could be contaminated if the closure flap was permeable and it would irrelevant as to whether or not the closure flap was open if the closure flap was permeable.

Roe et al. recites: "Methods of assaying insects for pesticide resistance and to identify insect species are based on feeding disruption caused by pesticides such as the biopesticide *Bacillus thuringiensis* toxin (Bt)." (Roe et al., Abstract, Lines 1-4). There is no correlation between a stool sample test and Roe et al. Roe et al. is cited solely for the proposition that allegedly cardboard is inherently permeable. In reality, stool sampling devices using cardboard flaps for receiving the stool specimen and applying a reagent must be impermeable. Otherwise, the test would be subject to contamination and ruined. Proof of this logical fact is replete in the United States Patent Office's own records. For example, U.S. Published Patent Application No. 2008/0113382 (hereinafter "Chandler" see Exhibit A) is a device for testing stool samples and recites: "A device and method for detecting the presence of hemoglobin in a biological sample, more particularly, the presence of blood in a fecal sample as an indicator of upper or lower gastrointestinal tract bleeding" (Chandler, Abstract, Lines 1-4). Chandler further recites: "The test strips were then placed in **a waterproof cardboard housing** (G) with a port (E) for sample and reagent addition and a window (F). For ease of reading the test result, the interface between pads B and C was located centrally in the observation window (F) of the test housing (G)" (emphasis added) (Chandler, Page 5, Paragraph [0093], Lines 7-12). Therefore, the cardboard housing for the stool sample testing device is impermeable.

Another example is U.S. Published Patent Application No. 2007/0092401 (hereinafter "Liao et al." see Exhibit B), which is a device for testing stool samples and recites: "The test device of the present invention can be used with an external collection slide 110. With reference to FIGS. 1-5, the collection slide 110 has a first

card 114 and a second card 112. The first and second cards may be made of any appropriate material. For example, the cards can be made of a **resilient, water resistant or water-impermeable material, such as plastic, coated cardboard**, metal, or glass. In one example, the cards are hingeably connected to each other, for example by a hinge 224 (FIG. 2)" (emphasis added) (Liao et al., Page 2, Paragraph [0021], Lines 1-9). Therefore, the cardboard flaps for the stool sample device is impermeable.

Still another example is U.S. Published Patent Application No. 2006/0246598 (hereinafter "Dai et al." see Exhibit C), which is a device for testing stool samples and recites: "The present invention provides collection slides for collecting a solid or semi-solid sample. In some embodiments, the sample is a biological sample, such as a stool sample. The present invention also provides devices for detecting the presence of analytes in the sample, and methods for collecting the sample. With reference to FIGS. 1-5, the collection slide 110 has a first card 114 and a second card 112. The first and second cards may be made of any appropriate material. For example, **the cards can be made of a resilient, water resistant or water-impermeable material**, such as plastic, **coated cardboard**, metal or glass" (emphasis added) (Dai et al., Page 3, Paragraph [0029], Lines 1-6 and Paragraph [0030], Lines 1-6). Therefore, the cardboard components functions as flaps for the stool sample device are impermeable.

Yet another example is U.S. Patent No. 7,488,450 (hereinafter "Matusewicz et al." see Exhibit D), which is a device for testing stool samples and recites: "A system for collecting biological samples, such as fecal specimens, and testing such samples for the presence of an analyte is disclosed" (Matusewicz et al., Abstract, Lines 1-3). Matusewicz et al. further recites: "FIGS. 1-3E illustrate the construction of a preferred embodiment of a sample collection device 10 having features of the present invention. A blank 12 is preferably die-cut or otherwise formed from a piece of rigid material as shown in FIG. 1. The rigid material is preferably a cellulose-based material that is **resistant to moisture** such as **cardboard**, paperboard and fiberboard, **the surfaces of**

which have been treated so as to make them moisture resistant, such as through the application of a varnish or laminate material" (emphasis added) (Matusewicz et al., Column 4, Lines 26-34). Once again, the cardboard structure for the stool sample testing device is constructed of **impermeable** cardboard.

Yet another example is U.S. Patent No. 6,077,711 (hereinafter "Singer" see Exhibit E), which is a device for testing stool samples and recites: "Referring now to the drawing, a frangible ampule specimen test card 10 is shown in FIGS. 1 and 2 and broadly includes a carrier 12, a frangible ampule 14, a channel 16, and at least one slide 18 for receiving a specimen such as a stool specimen thereon. The test card 10 as described herein is particularly useful in determining the presence of occult blood in a stool specimen" (Singer, Column 2, Lines 59-65). Singer further recites: "In greater detail, the carrier 12 may be formed of thin **cardboard or other protective and substantially non-permeable material** such as synthetic resin and preferably, presents a center web 26, a front side 28 and a back side 30" (emphasis added) (Singer, Column 3, Lines 12-15). As before, the cardboard cover for the stool sample testing device is constructed of an **impermeable** cardboard.

Another example is U.S. Patent No. 4,445,235 (hereinafter "Slover et al." see Exhibit F), which is a device for testing stool samples and recites: "This invention relates to a collector device for collection of a medical patient's stool specimen for examination and testing by a physician or a medical test laboratory" (Slover et al., Column 1, Lines 5-8). Slover et al. further recites: "Alternately, the receptacle can be constructed from a **cardboard material treated for water resistance**, which also permits biodegradability after expected duration of use" (emphasis added) (Slover et al., Column 2, Line 68 and Column 3, Lines 1-3). As before, the cardboard structure for the stool sample testing device is constructed of an **impermeable** cardboard.

Finally, another example is U.S. Patent No. 5,215,713 (hereinafter "Steinbiss" see Exhibit G), which is a device for testing stool samples and recites: "Test kit 10 for

determining an analyte in a pasty sample, in particular in stool." (Steinbiss, Abstract, Lines 1-2). Steinbiss further recites: "The first cover part 17 and the second cover part 18 possess in each case a recess 19 or 20, through which the fluid transport section is accessible in the eluant application zone 12 (suction layer 16b) or in the sample application zone 14 (sample layer 16a). The cover parts 17, 18 are made of a **moisture-proof layer material**, for example **coated cardboard** or a stable plastics film." (emphasis added) (Steinbiss, Column 4, Lines 13-19). As before, the flaps or cover parts for the stool sample testing device is constructed of an **impermeable** cardboard.

Consequently, the record is clear that stool sampling devices that are surrounded in cardboard require impermeable cardboard to protect the stool sample from contamination. It is erroneous to take permeable cardboard from a completely unrelated application, i.e., Roe et al., and try to make the case that all cardboard is inherently permeable when it would appear that all cardboard used to protect the stool sample and apply reagent in stool sampling devices is impermeable. Under 35 U.S.C. § 102, "the identical invention must be shown in as complete detail as is contained in the...claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 U.S.P.Q.2d 1913, 1920 (Fed. Cir. 1989). Moreover, when evaluating a claim for anticipation, all claim limitations must be considered. *In re Evanega*, 829 F.2d 1110, 4 U.S.P.Q.2d 1249 (Fed. Cir. 1987). It is clear from the evidence that not all cardboard is inherently permeable and that it would appear that the cardboard used in stool sampling kits are impermeable.

The requirement established by the Manual for Patent Examining Procedure (M.P.E.P. §2131.01) is that when there are multiple references used in a 35 U.S.C. § 102 rejection, the second reference must either:

1. show that the primary reference contains an "enabled disclosure" (which is not applicable);

2. explain the meaning of a term used in the primary reference (which is also not applicable); or
3. show that a characteristic not disclosed in the reference is inherent (**which is stated by the Examiner as being the reason**).

Such evidence must make clear that the missing descriptive matter is **necessarily present in the thing described in the reference**, and that it would be so recognized by persons of ordinary skill." (emphasis added) *Continental Can Co. USA v. Monsanto Co.*, 948 F.2d 1264, 1268, 20 U.S.P.Q.2d 1746, 1749 (Fed. Cir. 1991). It is respectfully believed that Applicant has proven that not all cardboard is inherently permeable and that the cardboard used in stool sampling devices are usually impermeable.

In addition, Hartl et al. does not disclose a malleable **material** securing strip but rather a slit. Applicant's Published Patent Application, i.e., U.S. Published Patent Application No. 20050112032, recites: "Again referring to FIG. 1, histological retaining device comprises a malleable securing strip 18. When extended flap portions 16a-d are folded to overlap target 14 (described in more detail below), **malleable securing strip 18 is designed to hold, crimp, or clamp the folded extended flap portions 16a-d to target 14; thereby, securing the extended flap portions 16a-d in the folded condition**. Additionally, malleable securing strip 18 provides positive release upon the opening of folded flap portions 16a-d after processing of the sample." (emphasis added) (Paragraph [0026], Lines 1-10). Also: "Malleable securing strip 18 can be **any material that is formable or malleable**, but it is **preferred that strip 18 is either a metal wire or a strip of heavy metal foil**. The **wire or foil needs to have appropriate dimensions to allow for a one time use-easy closure and clamping, as well as, positive release of extended flap portions 16a-d** (described in more detail below)." (emphasis added) (Paragraph [0028], Lines 1-7). Claims 1-4 are amended accordingly to provide the addition of the term "material" which provides a clear differentiation over a slit, which

is a void or absence of material. Numeral 18 in Hartl et al. is incorrectly identified by the Examiner as corresponding to the malleable securing strip. Hartl et al. recites: "Front sheet 11 can be covered with cover sheet 16 provided with tab 17 which is engageable with **slits 18 and 18'** in front sheet 11 and rear sheet 14 respectively" (Column 3, Lines 40-42). It is respectfully believed that a slit, which is an opening or void, is a very different item than a malleable securing strip. "The identical invention must be shown in as complete detail as is contained in the ... claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 U.S.P.Q.2d 1913, 1920 (Fed. Cir. 1989). It is respectfully believed that a slit is not identical to a malleable securing strip. An opening or void cannot function in the same manner as a solid object, i.e., malleable securing strip.

Therefore, it is respectfully believed that Claim 1 overcomes the rejection under 35 U.S.C. § 102(b) and is patentable over Hartl et al. in view of Roe et al. and is in condition for allowance.

Claim 2 depends from independent Claim 1, which are respectfully believed to overcome the 35 U.S.C. § 102(b) rejection over Hartl et al. in view of Roe et al. in the same manner as Claim 1 as described above. If an independent claim is not anticipated, then any claim depending therefrom is also not anticipated. In re Fine, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988).

Moreover, Claim 2 recites that the "...malleable securing strip is attached at an **edge** of the liquid permeable sheet." The slits 18 and 18' are not located at the edge of sheet but on the top face of the sheet as shown in FIGS. 1-3 of Hartl et al. **In fact, FIG. 3 clearly shows that both slits 18 and 18' are located a significant and appreciable distance from the edge of the permeable sheet.** When evaluating a claim for anticipation, all claim limitations must be considered. *In re Evanega*, 829 F.2d 1110, 4 U.S.P.Q.2d 1249 (Fed. Cir. 1987).

Therefore, it is respectfully believed that Claim 2 overcomes the rejection under 35 U.S.C. § 102(b) and is patentable over Hartl et al. in view of Roe et al. and is in condition for allowance.

Claim 6 depends from independent Claim 1, which are respectfully believed to overcome the 35 U.S.C. § 102(b) rejection over Hartl et al. in view of Roe et al. in the same manner as Claim 1 as described above. If an independent claim is not anticipated, then any claim depending therefrom is also not anticipated. In re Fine, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988).

Therefore, it is respectfully believed that Claim 6 overcomes the rejection under 35 U.S.C. § 102(b) and is patentable over Hartl et al. in view of Roe et al. and is in condition for allowance.

Rejection Under 35 U.S.C. § 103(a):

Claims 3 and 4 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Hartl et al. (U.S. Patent No. 4,225,557) in light of Roe et al. (U.S. Patent No. 6,060,039), as stated above and further in view of Rochette (U.S. Patent No. 3,537,636). Claims 3 and 4 depend from independent Claim 1 and are respectfully believed to overcome the rejection over Hartl et al. in light of Roe et al. in the same manner as Claim 1 as described above. If an independent claim is not obvious, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988).

As proven by the Applicant with regard to Claim 1, the cardboard used in stool sampling tests is impermeable. Accordingly, since the stool sampling patents and patent applications referenced in Exhibits A-G clearly and unequivocally state that the cardboard is impermeable, the premise that all cardboard is inherently permeable put forth in this Office Action is by necessity incorrect. The mere reference to "cardboard"

cannot imply permeability and when the prior art clearly indicates that when cardboard is used for a stool sampling device, the cardboard will need to be impermeable.

Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is **some teaching, suggestion, or motivation to do so**. *In re Kahn*, 441 F.3d 977, 986, 78 U.S.P.Q.2d 1329, 1335 (Fed. Cir. 2006). In this case, there is no teaching to combine cardboard used in a stool sampling device, which is clearly shown in the prior art as being impermeable with Roe et al., which discloses an insecticide resistance assay housed in cardboard. There is no reason to make this combination and no one with ordinary skill in the art would look to this reference to determine if stool sample testing devices should be made of permeable or impermeable cardboard. This person of ordinary skill in the art would look to comparable stool sample testing devices and come to the inescapable conclusion that cardboard is not inherently permeable but cardboard would need to be impermeable for this type of application.

Moreover, as stated previously, even if the slits 18 and 18′ disclosed in FIG. 3 of Hartl were replaced with a metal wire or metal foil, it would not be located at the edge of the permeable sheet, which is now required since Claims 3 and 4 now depend from Claim 2. In marked contrast, Rochette discloses: "...a strip of bendable inelastic material forming a longitudinal seam for the tubular bag...." (Rochette, Abstract, Lines 2-3). The bag is made of impermeable material, i.e., cellulose film (Rochette, Column 1, Line 65). This is only the combination of impermeable cardboard stool sample testing device found in Hartl with a bag closure for another impermeable container. It is respectfully believed to be axiomatic that features found in two references, i.e., malleable material securing strip, i.e., metal wire or metal foil, is attached at an **edge** of the **liquid permeable sheet** are not found in either of two cited references, they cannot come into being by their combination. **In reality, you would typically not add metal foil or a metal wire to a slit since it would make if more difficult to insert a**

cardboard flap into a slit if the slit has metal wire or metal foil around it. This is especially true for a slit that is not located at an edge. In this situation with the slit structure of Hartl, metal material would create more problems in that it would make it more difficult to engage the slit with a flap rather than provide reinforcement.

In determining obviousness, the proper analysis is whether the claimed invention would have been obvious to one of ordinary skill in the art after consideration of all the facts. "To reject a claim based on this rationale, U.S. Patent Office personnel must resolve the Graham factual inquiries. Office personnel must then articulate the following: (1) **a finding that the prior art included each element claimed**, although not necessarily in a single prior art reference, **with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference**; (2) a finding that one of ordinary skill in the art could have combined the elements as claimed by known methods, and that in combination, each element merely would have performed the same function as it did separately." (emphasis added) (Federal Register / Volume 72, No. 195 / Wednesday, October 10, 2007 / Notices, Page 57529, *"Examination Guidelines for Determining Obviousness Under 35 U.S.C. § 103 in View of the Supreme Court Decision in KSR International Co. v. Teleflex Inc."*) (emphasis added). It is respectfully believed that it is very clear that this rejection completely fails the new KSR Guidelines promulgated by the United States Patent Office since the cardboard found in Hartl or other stool sample testing devices is not permeable and the malleable securing strip is not located at the edge of the permeable sheet.

Therefore, it is respectfully believed that Claims 3 and 4 overcome the rejection under 35 U.S.C. § 103(a) over Hartl et al. in light of Roe et al., as stated above, and further in view of Rochette and are in condition for allowance.

Claim 5 was rejected under 35 U.S.C. § 103(a) as being unpatentable over Hartl et al. (U.S. Patent No. 4,225,557) in light of Roe et al. (U.S. Patent No. 6,060,039), as

stated above and further in view of Rochette (U.S. Patent No. 3,537,636) and in view of Lee (U.S. Patent No. 6,372,514). Claim 5 depends from independent Claim 1 and is respectfully believed to overcome the rejection over Hartl et al. in light of Roe et al. in the same manner as Claim 1 as described above. If an independent claim is not obvious, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988).

As proven by the Applicant with regard to Claim 1, the cardboard used in stool sampling tests is impermeable. Accordingly, since the stool sampling patents and patent applications referenced in Exhibits A-G clearly and unequivocally state that the cardboard is impermeable, the premise that all cardboard is inherently permeable put forth in this Office Action is by necessity incorrect. The mere reference to "cardboard" cannot not imply permeability and when the prior art clearly indicates that when cardboard is used for a stool sampling device, the cardboard needs to be impermeable.

Lee is cited for disclosing a chromatographic strip that is coated with gelatin to "enhance the life of the strip and clarity of any visible reactions produced in the test." (Office Action, Page 4, Lines 20-21). This is a totally different function and purpose than the release agent disclosed in the Applicant's Patent Application, which is for: "Additionally, the chipboard card stock is preferably coated with a release agent to assist in forming the separation of the card from its wax impregnated specimen--to be investment cast into the wax block." (Applicant's U.S. Published Patent Application No. 2005/0112032, Paragraph [0023], Lines 5-8). The removal of a wax impregnated tissue specimen from the target directly conflicts with the purpose stated in the Office Action on Page 5, Lines 1-5, which states: "At the time of the invention, it would have been obvious to a person of ordinary skill in the art to modify Hartl, in light of Roe, by incorporating gelatin to the liquid permeable (sic) target of Hartl, in light of Roe, because enhance the life of the device and clarity of any visible reactions produced in the test...." As clearly shown in the Applicant's Published Patent Application, this is

not the reason that gelatin was used by the Applicant so that an individual of ordinary skill in the art would not be motivated to utilize gelatin disclosed in Lee with the stool sample device found in Hartl and this is clearly refuted by the Applicant's own patent application. Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so. *In re Kahn*, 441 F.3d 977, 986, 78 U.S.P.Q.2d 1329, 1335 (Fed. Cir. 2006).

"To reject a claim based on this rationale, U.S. Patent Office personnel must resolve the Graham factual inquiries. Office personnel must then articulate the following: (1) a finding that the prior art included each element claimed, although not necessarily in a single prior art reference, with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference; (2) a finding that one of ordinary skill in the art could have combined the elements as claimed by known methods, and that in combination, **each element merely would have performed the same function as it did separately**" (Federal Register / Volume 72, No. 195 / Wednesday, October 10, 2007 / Notices, Page 57529, "*Examination Guidelines for Determining Obviousness Under 35 U.S.C. § 103 in View of the Supreme Court Decision in KSR International Co. v. Teleflex Inc.*") (emphasis added). It is respectfully believed that it is very clear that this rejection completely fails the new KSR Guidelines promulgated by the United States Patent Office since the use of gelatin in Lee has no bearing on the use of gelatin in the Applicant's claimed Invention. "In determining the propriety of the Patent Office case for obviousness in the first instance, it is necessary to ascertain whether or not the reference teachings would appear to be sufficient for one of ordinary skill in the relevant art having the reference before him to make the proposed substitution, combination, or other modification." *In re Linter*, 458 F.2d 1013, 1016, 173 U.S.P.Q. 560, 562 (C.C.P.A. 1972).

Therefore, it is respectfully believed that Claim 5 overcomes the rejection under 35 U.S.C. § 103(a) over Hartl et al. in light of Roe et al., as stated above, and further in view of Lee and is in condition for allowance.

Claim 7 was rejected under 35 U.S.C. § 103 (a) as being unpatentable over Hartl et al. (U.S. Patent No. 4,225,557) in view of Roe et al. (U.S. Patent No. 6,060,039). Claim 7 depends from independent Claim 1 and is respectfully believed to overcome the rejection over Hartl et al. in light of Roe et al. in the same manner as Claim 1 as described above. If an independent claim is not obvious, then any claim depending therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988).

As proven by the Applicant with regard to Claim 1, the cardboard used in stool sampling tests is impermeable. Accordingly, since the stool sampling patents and patent applications referenced in Exhibits A-G clearly and unequivocally state that the cardboard is impermeable, the premise that all cardboard is inherently permeable put forth in this Office Action is by necessity incorrect. The mere reference to "cardboard" cannot not imply permeability and when the prior art clearly indicates that when cardboard is used for a stool sampling device, the cardboard needs to be impermeable.

Moreover, it is acknowledged that the use of X and Y coordinate lines are completely absent from both Hartl et al. and Roe et al. In determining obviousness, the proper analysis is whether the claimed invention would have been obvious to one of ordinary skill in the art after consideration of all the facts. "To reject a claim based on this rationale, U.S. Patent Office personnel must resolve the Graham factual inquiries. Office personnel must then articulate the following: (1) **a finding that the prior art included each element claimed**, although not necessarily in a single prior art reference, **with the only difference between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference**; (2) a finding that one of ordinary skill in the art could have combined the elements as

claimed by known methods, and that in combination, each element merely would have performed the same function as it did separately." (emphasis added) (Federal Register / Volume 72, No. 195 / Wednesday, October 10, 2007 / Notices, Page 57529, *"Examination Guidelines for Determining Obviousness Under 35 U.S.C. § 103 in View of the Supreme Court Decision in KSR International Co. v. Teleflex Inc."*) (emphasis added). It is respectfully believed that it is very clear that this rejection completely fails the new KSR Guidelines promulgated by the United States Patent Office since the X and Y coordinate markings are wholly absent from both Hartl et al. and Roe et al. It is respectfully believed that a feature that is absent from both cited References cannot come into being by their combination.

Moreover, a statement that modifications of the prior art to meet the claimed invention would have been "well within the ordinary skill of the art at the time the claimed invention was made" because the references relied upon teach that all aspects of the claimed invention were individually known in the art is **not sufficient** to establish a prima facie case of obviousness without some objective reason to combine the teachings of the references. *Ex parte Levengood*, 28 U.S.P.Q.2d 1300 (Bd. Pat. App. & Inter. 1993). **"[R]ejections on obviousness cannot be sustained by mere conclusory statements**; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." (emphasis added) *In re Kahn*, 441 F.3d 977, 988, 78 U.S.P.Q.2d 1329, 1336 (Fed. Cir. 2006). Manual for Examining Procedure (M.P.E.P. §2143.01 IV). It was explicitly held in *In re Sang Sung Lee*, 61 U.S.P.Q.2d 1430 (Fed. Cir. 2002), that rejection of patent application must be based on evidence comprehended by language of that section, and search for and analysis of prior art includes evidence relevant to finding of whether there is teaching, motivation, or suggestion to modify a reference, and the Board of Patent Appeals and Interferences must explain reasons why one of ordinary skill in art would have been motivated to select a reference and to modify it. In this case, it is

respectfully believed that there is no objective evidence of record that would lead an individual of ordinary skill in the art to modify Hartl.

The Office Action on Page 5, Lines 10-15 states that: "It would have been obvious to a person of ordinary skill in the art to modify Hartl, in light of Roe, by physically having the X and Y coordinate marking lines on the liquid permeable target because it would be (sic) allow the user to quickly determine whether the sample is placed on the portions of the target that has the greatest color intensity to insure a correct diagnosis is made." This is directly refuted by Hartl, which recites: "Providing that developer is applied on the reverse side of layer 13 to those areas thereof which correspond on the front side to the intersection of the diagonals A-D and B-C, the sites of greatest color intensity on development will lie in the direction of these diagonals A-D and B-C, shown in dotted lines in FIG. 1 for explanatory purposes" (Hartl, Column 4, Lines 23-29). Therefore, Hartl clearly states that the greatest color intensity will be along diagonal lines so that the X and Y coordinates would not provide the greatest color intensity. Therefore, this reasoning providing in the Office Action is incorrect and directly refuted by the express language of Hartl. Moreover, the reason that Applicant's claimed invention requires X and Y coordinate is stated as follows: "Fourth, permeable target 14 may also contain a printed X/Y coordinate marking system 20 centered on target 14 as shown in FIG. 1. Marking system 20 consists of horizontal and vertical marking lines 22a and 22b along with measurement marking lines 24 spaced at equal distances on horizontal and vertical marking lines 22a and 22b. Any spacing of measurement marking lines 24 is appropriate, but 1 mm spacing is preferred. X/Y coordinate marking system 20 is designed to allow for proper orientation of a tissue sample when placed on target 14 and serve as a scalable reference for optional photographic imaging of the tissue specimen." (Applicant's Published Patent Application No. 2005/0112032, Paragraph [0025], Lines 1-12). Therefore, a coordinate system for photographic imaging has nothing to do with color

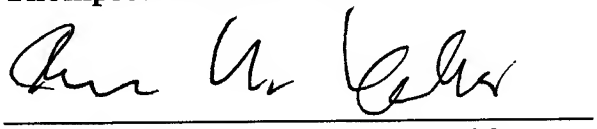
reactions involving a stool sample. If any lines were added to the invention of Hartl, it must be along the diagonal rather than the X and Y axis since the Office Action states that the lines are used highlight the portions of the target with the greatest color intensity. Therefore, a person of ordinary skill in the art would be instructed to only add diagonal lines and not lines along the X and the Y axis. Moreover, it would not achieve the Applicant's objective of providing a coordinate system for photographic imaging. The Supreme Court held in *U.S. v. Adams*, 383 U.S. 39, 148 U.S.P.Q. 479 (1966), that one important indicium of nonobviousness is "teaching away" from the claimed invention by the prior art or by experts in the art at (and/or after) the time the invention was made. This is specifically mandated by the Manual of Patent Examining Procedure (M.P.E.P.) § 2141.02. It is respectfully believed that a citation of prior art references that teaches, based on the Examiner's analysis, that lines along the diagonal are needed to provide the greatest color intensity so that an X and a Y coordinate system is undesirable would provide strong evidence as to the patentability of the Applicant's Invention. This is in addition to the fact that the X and Y coordinate system in Applicant's Invention is used for an entirely different purpose.

Therefore, it is respectfully believed that Claim 7 overcomes the rejection under 35 U.S.C. § 103 (a) and is patentable over Hartl et al. in view of Roe et al. and is in condition for allowance.

Therefore, it is now believed that all of the pending Claims, i.e., Claims 1-7, in the present application are in condition for allowance. Favorable action and allowance of the Claims is therefore respectfully requested. If any issue regarding allowability of any of the pending Claims in the present application could be readily resolved, or if other action could be taken to further advance this application such as an Examiner's Amendment, or if the Examiner should have any questions regarding the present Amendment, it is respectfully requested that the Examiner please telephone the Applicant's undersigned attorney in this regard.

Respectfully submitted,

Thompson Coburn LLP

By: 

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Appendix

Exhibit A

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Chandler

(54) **DEVICE AND METHOD FOR DETECTING THE PRESENCE OF HEMOGLOBIN IN A BIOLOGICAL SAMPLE**

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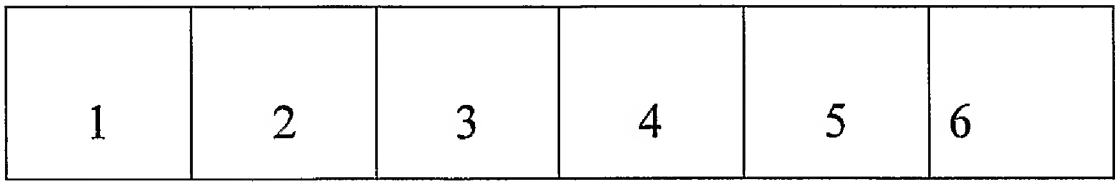
(57) **ABSTRACT**

A device and method for detecting the presence of hemoglobin in a biological sample, more particularly, the presence of blood in a fecal sample as an indicator of upper or lower gastrointestinal tract bleeding.



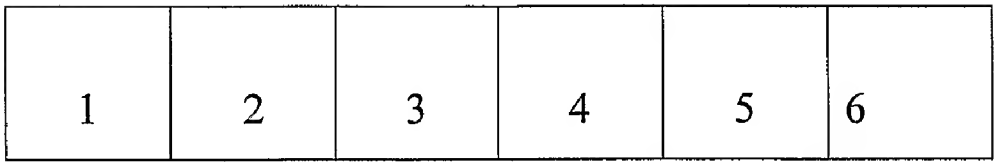


Figure 1

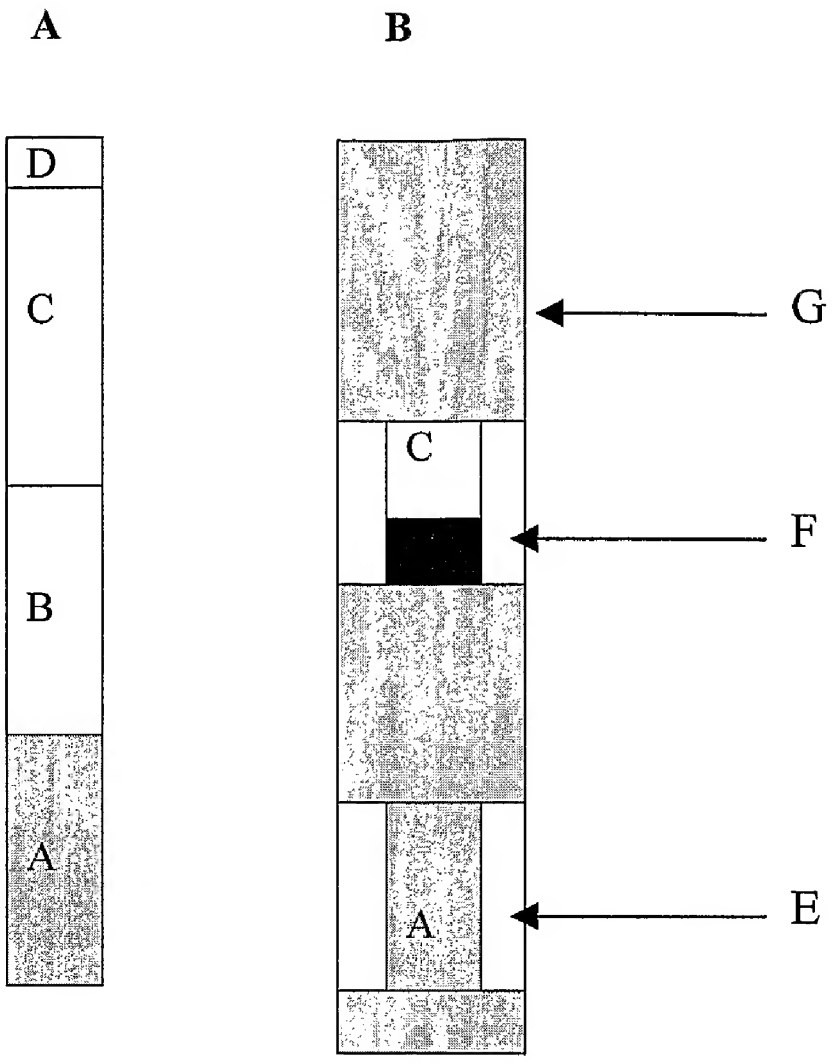


Figure 2

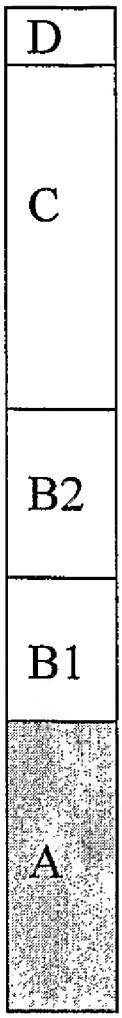


Figure 3

DEVICE AND METHOD FOR DETECTING THE PRESENCE OF HEMOGLOBIN IN A BIOLOGICAL SAMPLE

FIELD OF THE INVENTION

[0001] The present invention relates generally to a device and method for detecting the presence of hemoglobin in a biological sample. More particularly, the present invention provides a device and method for detecting the presence of blood in a biological sample and still more particularly, the presence of blood in a fecal sample as an indicator of upper or lower gastrointestinal tract bleeding. The method of the present invention is useful, inter alia, for the diagnosis of gastrointestinal tract diseases which can be detected by detecting intestinal bleeding.

BACKGROUND OF THE INVENTION

[0002] Bleeding into the bowel is currently the best early indicator of bowel cancer (also know as colorectal cancer). Testing for symptoms of bleeding into the bowel is usually achieved by screening stools for the presence of blood. This test is often referred to as fecal occult blood testing (referred to as "FOBT").

[0003] Chemical tests are most widely used for FOBT. These tests typically require stool to be applied to paper impregnated with a chromogen indicator, such as guaiac or 3,3',5,5'-tetramethylbenzidine (TMB), which changes color on oxidation. When developer solution is added to the paper, a blue color develops with a positive result. Guaiac tests have the advantage of being inexpensive and easy to perform, but are less accurate (not specific for human blood) and less sensitive than desirable. Nevertheless, several international studies have shown that screening patients with these tests can save lives through the early detection of pre-cancerous and cancerous lesions. The commonly used guaiac tests detect the heme of hemoglobin, and as this is relatively resistant to breakdown in the small intestine, these tests may detect bleeding anywhere within the intestinal tract. For colorectal cancer screening this may be a disadvantage as these tumors are confined to the large intestine.

[0004] Recently, more sensitive and specific immunological tests (e.g. immunochromatographic tests) have been developed that have the potential to improve the accuracy of detecting blood in screening for colorectal cancer. These tests typically detect the globin protein of hemoglobin, a protein that does not survive passage through the upper gastrointestinal tract. A positive immunological test therefore indicates lower gastrointestinal bleeding. In common with all immunologically based tests, however, these tests are subject to a "prozone" or "high dose hook" effect, where at high levels of analyte, the test may be inhibited to the extent that heavy bleeding may be missed.

[0005] Heme from hemoglobin has a pseudoperoxidase activity that catalyses the breakdown of peroxide substrates and the release of oxygen. The released oxygen may be detected by suitable chromogenic indicators such as guaiac and tetramethylbenzidine (TMB) which change color on oxidation. Fecal Occult Blood Tests (FOBTs) detect intestinal bleeding by use of this reaction to detect heme from the hemoglobin of red blood cells, and a variety of formats for such tests are known in the art (see, for example, U.S. Pat. Nos. 3,996,007; 4,225,557; 4,789,629; 5,064,766; 5,100,619; 5,106,582; 5,171,528; 5,171,529 and 5,182,191). Typically, FOBTs involve smearing a stool sample on guaiac-impregnated paper and adding a developer solution containing peroxide. If heme is present, a blue color develops on or around the stool specimen. The disadvantages of these tests include:

[0006] the stool sample may also contain peroxidases or pseudoperoxidases from ingested foods and these may cause a (false) positive reaction in the absence of human blood from the intestinal tract;

[0007] heme from ingested meat may also cause a false positive reaction;

[0008] the blue color developed with a positive test must be read against a dark background of stool, so that at lower heme concentrations the result may be equivocal;

[0009] with a positive result, color diffuses away from the stool sample, becomes weaker in intensity, and may fade out (the transitory nature of the color change may make also make interpretation of the test result difficult or unreliable);

[0010] the developer solution, containing peroxide and other reagents, can interfere with immunochemical tests that may otherwise be used in conjunction with this test for differentiation between upper and lower gastrointestinal bleeding (see, for example, International Patent Publication WO 00/29852, Enterix Inc., combining a chromogen test to detect any intestinal bleeding and an immunochemical test to detect lower intestinal bleeding only).

[0011] FOBTs have also been described that have a peroxide reagent such as cumene hydroperoxide dried in a paper matrix (see, for example, Lam, U.S. Pat. No: 4,071,318). In this case, the test paper can be added directly to water and will develop color if heme is present in the water. These FOBTs are typically added to a toilet bowl containing a stool after a bowel movement in order to detect blood released from the stool into the water. The disadvantages of these tests include:

[0012] blood on, or in, the stool may not diffuse into the water in sufficient concentration to allow detection;

[0013] the test must be read against a background of stool and toilet paper, making interpretation difficult;

[0014] the tests may also be subject to interference from dietary heme or peroxidases if there is direct contact between the stool and test paper;

[0015] the undeveloped test papers must be stored after manufacture in desiccated conditions to prevent breakdown of the peroxide reagent and development of color in the test paper.

SUMMARY OF THE INVENTION

[0016] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0017] In one aspect, the present invention provides a device for use in the detection of hemoglobin in a biological sample, particularly a fecal sample, comprising a carrier matrix which includes:

[0018] (i) a sample application region for receipt of said biological sample;

[0019] (ii) a substrate region in liquid-conductive communication with, or combined with, the sample application region and having a pseudoperoxidase substrate

applied thereto or impregnated therein, said pseudoperoxidase substrate comprising a peroxide or hydroperoxide reagent; and

[0020] (iii) an indicator region in liquid-conductive communication with, or combined with, the substrate region and having an indicator applied thereto or impregnated therein, said indicator producing a detectable response in the presence of heme and said pseudoperoxidase substrate.

[0021] In one embodiment of this aspect of the invention, the sample application region and the substrate region may be combined into a single, combined sample application/substrate region having the pseudoperoxidase substrate applied thereto or impregnated therein. In the preferred embodiment, however, the sample application region and the substrate region are separate regions of the carrier matrix which are in liquid-conductive communication.

[0022] In another embodiment, the substrate region and the indicator region may be combined into a single, combined substrate/indicator region having both the pseudoperoxidase substrate and the indicator applied thereto or impregnated therein. Preferably, however, the substrate region and the indicator region are separate regions of the carrier matrix which are in liquid-conductive communication.

[0023] In another aspect, the present invention provides a method for the detection of hemoglobin in a biological sample, particularly a fecal sample, comprising the steps of:

[0024] (i) applying said biological sample to a sample application region of a carrier matrix which comprises said sample application region, a substrate region and an indicator region;

[0025] (ii) contacting said biological sample with the substrate region wherein said sample is contacted with a pseudoperoxidase substrate comprising a peroxidase or hydroperoxidase reagent; and

[0026] (iii) contacting said sample and pseudoperoxidase substrate with the indicator region wherein said sample and substrate are contacted with an indicator which produces a detectable response in the presence of heme and said pseudoperoxidase substrate.

[0027] In one embodiment of this aspect of the invention, the biological sample may be contacted with the pseudoperoxidase substrate in a single, combined sample application/substrate region of the carrier matrix, before permitting or causing flow of the sample and substrate to the indicator region. In a preferred embodiment, however, the sample application region and the substrate region are separate regions of the carrier matrix which are in liquid-conductive communication, and the biological sample is applied to the sample application region before permitting or causing flow of the sample to the substrate region.

[0028] In another embodiment the biological sample may be contacted with the pseudoperoxidase substrate and indicator in a single, combined substrate/indicator region of the carrier matrix, by permitting or causing flow of the sample from the sample application region to the substrate/indicator region. In a preferred embodiment, however, the substrate region and the indicator region are separate regions of the carrier matrix which are in liquid-conductive communication, and the biological sample is applied to the sample application region before permitting or causing flow of the sample to the substrate region, and then permitting or causing flow of the sample and substrate to the indicator region.

[0029] In a particularly preferred embodiment, the device and method of the present invention may combine the detection of heme in a biological sample as broadly outlined above, with an immunochemical test for the detection of globin, thereby providing a dual test for differentiation between upper and lower gastrointestinal tract bleeding which is particularly useful for the detection of lower gastrointestinal tract diseases such as colorectal cancer.

[0030] In this preferred embodiment, the carrier matrix of the device as broadly described above further comprises:

[0031] (iv) a second substrate region in liquid-conductive communication with the sample application region and having a detectable antiglobin immunointeractive molecule applied thereto or impregnated therein, said immunointeractive molecule forming a detectable globin-antiglobin complex in the presence of globin; and

[0032] (v) a detection region in liquid-conductive communication with the second substrate region and having an anti-globin immunointeractive molecule immobilized therein, said immobilized immunointeractive molecule immobilizing said detectable globin-antiglobin complex.

[0033] Similarly, in this preferred embodiment, the method of the present invention as broadly described above further comprises the steps of:

[0034] (vi) contacting said biological sample with a second substrate region wherein said sample is contacted with a detectable antiglobin immunointeractive molecule to form a detectable globin-antiglobin complex in the presence of globin; and

[0035] (vii) contacting said detectable globin-antiglobin complex with a detection region wherein said detectable globin-antiglobin complex is contacted with an immobilized antiglobin immunointeractive molecule to immobilize said detectable globin-antiglobin complex.

[0036] Preferably, the biological sample is permitted or caused to flow from the sample application region to the second substrate region which is in liquid-conductive communication with the sample application region, and the detectable globin-antiglobin complex is permitted or caused to flow from the second substrate region to the detection region which is in liquid-conductive communication with the second substrate region.

DETAILED DESCRIPTION OF THE INVENTION

[0037] The present invention provides an improved test format for the detection of hemoglobin which is particularly suitable for the detection of hemoglobin as an indicator of intestinal bleeding. The format is also designed to be compatible with immunochemical tests for the detection of globin so that a dual test for differentiation between upper and lower intestinal bleeding is feasible.

[0038] The invention involves the use of lateral flow of a liquid sample (suspected of containing blood) from a point of application through one or more regions containing the reagents required for detection of the heme. The lateral flow layout of the components of the test format has the following advantages:

[0039] the color produced with a positive result accumulates as the flow reaches the end of the carrier matrix, concentrating the color and facilitating the ease of reading;

[0040] the color of a positive reaction is free of any fecal or other obscuring background material;

[0041] dietary contaminants, such as heme from meat or peroxidases from food, are diluted on lateral flow, in many cases to below their threshold detection level;

[0042] the sample application region may contain enhancing agents that promote accurate and sensitive detection of the heme by the downstream test components;

[0043] incompatible or mutually unstable components or reagents (e.g. substrate and indicator reagents) may be located in separate regions, allowing long-term storage without special manufacturing or storage precautions;

[0044] as all reagents can be impregnated in the separate regions, water, water/ethanol mixtures or any other inert reagent may be used for the test development;

[0045] the chromogenic heme test (which detects any intestinal bleeding) may therefore be combined with an immunochemical test (which is specific for lower intestinal bleeding) so as to allow for discrimination between upper and lower intestinal bleeding on the one test sample.

[0046] The present invention provides a test device for use in the detection of hemoglobin in a biological sample, particularly a fecal sample, comprising a carrier matrix which includes:

[0047] (i) a sample application region for receipt of said biological sample;

[0048] (ii) a substrate region in liquid-conductive communication with, or combined with, the sample application region and having a pseudoperoxidase substrate applied thereto or impregnated therein, said pseudoperoxidase substrate comprising a peroxide or hydroperoxide reagent; and

[0049] (iii) an indicator region in liquid-conductive communication with, or combined with, the substrate region and having an indicator applied thereto or impregnated therein, said indicator producing a detectable response in the presence of heme and said pseudoperoxidase substrate.

[0050] The invention also provides a test method for the detection of hemoglobin in a biological sample, particularly a fecal sample, comprising the steps of:

[0051] (i) applying said biological sample to a sample application region of a carrier matrix which comprises said sample application region, a substrate region and an indicator region;

[0052] (ii) contacting said biological sample with the substrate region wherein said sample is contacted with a pseudoperoxidase substrate comprising a peroxidase or hydroperoxidase reagent; and

[0053] (iii) contacting said sample and pseudoperoxidase substrate with the indicator region wherein said sample and substrate are contacted with an indicator which produces a detectable response in the presence of heme and said pseudoperoxidase substrate.

[0054] Reference to a "biological sample" should be understood as a reference to any sample of biological material derived from an animal such as, but not limited to, faeces, mucus, urine, biopsy specimens and fluid which has been introduced into the body of an animal and subsequently removed such as, for example, the saline solution extracted from the lung following lung lavage or the solution retrieved from an enema wash. The biological sample which is tested according to the method of the present invention may be tested directly or may require some form of treatment prior to testing. For example, a biopsy sample may require homogenisation prior to testing. Further, to the extent that the biological sample is not in liquid form, (for example it may be a solid, semi-solid or dehydrated liquid sample) it may require the addition of a reagent, such as a buffer, to mobilize the sample. The mobilizing reagent may be mixed with the biological sample prior to application of the sample to the carrier matrix or the reagent may be applied to the sample after the sample has been applied to the carrier matrix. The use of a mobilizing reagent may also be required to facilitate lateral flow (wicking) of the sample along the carrier matrix. Preferably, the biological sample is a gastrointestinal sample. By "gastrointestinal sample" is meant any sample which is derived from the gastrointestinal tract. For example, feces, mucus (for example the mucus from a rectal mucus swab), enema wash solution or a gastrointestinal tract biopsy sample. Most preferably, the biological sample is a stool sample, or a sample of water from a toilet bowl containing a stool.

[0055] The term "animal" as used herein includes a human, primate, livestock animal (e.g. sheep, pig, cow, horse, donkey), laboratory test animal (e.g. mouse, rat, rabbit, guinea pig) companion animal (e.g. dog, cat), captive wild animal (e.g. fox, kangaroo, deer), aves (e.g. chicken, geese, duck, emu, ostrich), reptile or fish. Preferably, however, the animal is a human.

[0056] Preferably, the carrier matrix used in forming the test device of the present invention is in the form of a test strip of a suitable material which permits liquid-conductive communication between the various zones or regions of the matrix. A particularly preferred material is one which allows capillary flow, such as an open-celled, chemically inert matrix, with porous plastics material, filter paper and glass fiber being preferred. Other suitable materials are well known in the art (see, for example, Lam U.S. Pat. No. 4,071,318), and are intended to be encompassed within the scope of the present invention.

[0057] Preferably, the sample application region of the carrier matrix includes an absorbent pad such as a non-woven polyester pad which is impregnated with a reagent to lyse any red blood cells present in the sample so as to release hemoglobin, to minimise binding to the pad and to promote sample flow from the pad. Particularly suitable lysis reagents are detergents (such as Triton X100). After the sample is applied to the pad, a mobilizing agent such as water or a water/ethanol mixture can be applied to the pad to mobilize any heme (and globin) in the sample and permit or cause the sample to flow or wick by capillary action through the carrier matrix to the substrate region(s).

[0058] The pseudoperoxidase substrate which is present in the substrate region comprises a peroxidase or hydroperoxidase reagent as the main reagent, optionally together with supplementary stabilizers, enhancers and accelerators which are known to persons skilled in the art. Suitable peroxidase or hydroperoxidase reagents include, for example, t-butyl hydroperoxide, cumene hydroperoxide, diisopropylbenzene hydroperoxide, 2,5-dimethylhexane-2,5-dihydroperoxide, paramenthane hydroperoxide or mixtures thereof. Of these, cumene hydroperoxide has been found to be most preferable. Suitable stablilizing and enhancing agents are also well known in the art, and include borate esters such as trimethanolamine borate, triethanolamine borate and tri(n-propanol)

amine borate, as stabilizing agents, and 6-methoxyquinoline as an enhancing agent (see Lam U.S. Pat. No. 4,071,318).

[0059] The indicator which is present in the indicator region to produce a detectable response in the presence of heme and the pseudoperoxidase substrate is preferable a chromogen such as guaiac or a benzidine compound, for example benzidine, o-tolidine, 3,3',5,5'-tetramethylbenzidine (TMB), 2,7-diaminofluorene, or mixtures of these in varying proportions. Once again, stabilizing agents and/or enhancing agents which are well known to persons skilled in the art may be included in the indicator.

[0060] Preferably, in carrying out the method of the present invention, the sample is applied to an absorbent sample pad in the sample application region where any red blood cells in the sample are lysed by detergent or other lysis reagent in the sample pad to release the hemoglobin. The sample is then permitted or caused to flow by capillary action from the sample pad to the substrate region using water or water/ethanol as a mobilizing agent to develop the test and to solubilize dried pseudoperoxidase substrate (such as cumene peroxide) and other stabilizing and enhancing reagents located in this substrate region. The sample and pseudoperoxidase substrate are then permitted or caused to flow by capillary action to the indicator region where the presence of heme in the sample is detected by a reaction with the chromogen such as guaiac or TMB, resulting in a detectable color change.

[0061] In a preferred aspect of the invention, the detection of heme in the biological sample, as described in detail above, is combined with an immunochemical test in a "dual test" which allows differentiation of upper and lower intestinal bleeding in the test sample.

[0062] Accordingly, in this aspect, the present invention provides a test device as broadly described above in which the carrier matrix further comprises:

[0063] (iv) a second substrate region in liquid-conductive communication with the sample application region and having a detectable antiglobin immunointeractive molecule applied thereto or impregnated therein, said immunointeractive molecule forming a detectable globin-antiglobin complex in the presence of globin; and

[0064] (v) a detection region in liquid-conductive communication with the second substrate region and having an anti-globin immunointeractive molecule immobilized therein, said immobilized immunointeractive molecule immobilizing said detectable globin-antiglobin complex.

[0065] The carrier matrix of the test device of the present invention may also comprise additional regions to the regions specifically described above. For example, the device may also comprise an absorbent pad or pads located after the indicator region and/or detection region to draw the mobilising liquid front from the sample application region through the respective regions in liquid-conductive communication with each other in order to develop the tests.

[0066] In the preferred aspect, the present invention also provides a method as broadly outlined above which further comprises the steps of:

[0067] (vi) contacting said biological sample with a second substrate region wherein said sample is contacted with a detectable antiglobin immunointeractive molecule to form a detectable globin-antiglobin complex in the presence of globin; and

[0068] (vii) contacting said detectable globin-antiglobin complex with a detection region wherein said detectable globin-antiglobin complex is contacted with an immobilized antiglobin immunointeractive molecule to immobilize said detectable globin-antiglobin complex.

[0069] Reference throughout this specification to "immunointeractive molecule" should be understood as a reference to any molecule comprising an antigen binding portion or a derivative of said molecule. Examples of molecules contemplated by this aspect of the present invention include, but are not limited to, monoclonal and polyclonal antibodies (including synthetic antibodies), hybrid antibodies, humanised antibodies, catalytic antibodies) and T cell antigen binding molecules. Preferably, said immunointeractive molecule is an antibody.

[0070] Full details of suitable detectable antiglobin immunointeractive molecules present in the second substrate region, and of suitable immobilised antiglobin immunointeractive molecules present in the detection region, are set out in International Patent Publication No. WO 00/29852, in the name of Enterix Inc., the contents of which are incorporated herein by reference.

[0071] "Detecting" the formation of a globin-antiglobin complex may be by any convenient method which will be known to those skilled in the art. In the preferred method of the invention described herein, the antiglobin antibody which becomes resuspended by the wicking biological sample front is complexed with colloidal gold. As the globin-antiglobin/colloidal gold complex is trapped by the antiglobin capture antibody impregnated in the detection region of the carrier matrix, the colloidal gold becomes visible as a pink band due to its increasing concentration during trapping of the complex at this point. Alternatively, the antiglobin antibody may be radio-labeled, or enzymatically labeled such that upon addition of a substrate a color change is observed if globin is present.

[0072] In one preferred embodiment of the "dual test" aspect of the present invention, detection of heme is carried out as described in detail above. Detection of globin in the biological sample is carried out using a chromatographic test strip which comprises a second substrate region and a detection region. The second substrate region is an area of immobilized antiglobin antibody coupled to colloidal gold particles which are resuspendible by a passing liquid front, while the detection region is an area of immobilized antiglobin capture antibody.

[0073] In this preferred aspect of the invention, the biological sample which is applied to the sample application region flows or wicks to the second substrate region and at this region, the globin component of any hemoglobin which is present in the sample is bound by the antiglobin antibody coupled to the colloidal gold particles. The passing biological sample front re-suspends these antibodies and the globin-antiglobin complex flows or wicks to the detection region where the globin component of any hemoglobin present in the sample and bound in the globin-antiglobin complex becomes bound to the immobilized antiglobin capture antibody where it is detectable.

[0074] In this dual test aspect, the present invention may be used to diagnose gastrointestinal tract bleeding by analysing fecal samples for the presence of blood. Without limiting the present invention to any one theory or mode of action, the chromogen test will positively identify bleeding from any part of the gastrointestinal tract (that is, both the upper and

lower regions of the tract) since it detects the heme component of hemoglobin and heme is relatively resistant to breakdown in the small intestine (the upper gastrointestinal tract). The globin component of hemoglobin however, does not survive passage through the upper gastrointestinal tract. A positive globin result in a fecal sample therefore indicates that bleeding has occurred in the lower gastrointestinal tract. Accordingly, by applying a combined dual immunological and non-immunological based test, it is possible to differentiate between upper and lower gastrointestinal tract bleeding wherein a positive heme result together with a negative globin result indicates upper gastrointestinal tract bleeding, and a positive heme result together with a positive globin result indicates lower gastrointestinal tract bleeding. This is of particular importance, for example to the diagnosis of colorectal cancer, the symptoms of which include lower gastrointestinal tract bleeding.

[0075] Further features of the test device and method of the present invention are more fully described below with reference to the accompanying drawing.

BRIEF DESCRIPTION OF THE DRAWINGS

[0076] FIG. **1** is a schematic representation of a "dual test" device in accordance with a preferred embodiment of the present invention.

[0077] FIGS. **2**A and **2**B are schematic representations of an alternative device in accordance with the present invention. FIG. **2**A shows a FOBT test strip, and FIG. **2**B shows a FOBT test strip in housing.

[0078] FIG. **3** is a schematic representation of a further alternative device in accordance with the present invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

[0079] FIG. **1** shows schematically a dual test strip format in accordance with the present invention, which comprises the following components in liquid-conductive communication in a single test strip, as follows:

[0080] TMB impregnated indicator paper (**1**);

[0081] Substrate (e.g. dried cumene peroxide) impregnated paper (**2**);

[0082] Sample pad (e.g. non woven polyester impregnated detergent, e.g. with Triton X100) (**3**);

[0083] Conjugate pad (e.g. gold labeled anti-human globin antibodies) (**4**);

[0084] Solid phase (e.g. nitrocellulose membrane with immobilized anti-human globin antibody line and a procedural control line) (**5**);

[0085] Absorbent pad (**6**).

[0086] Components **1**, **2** and **3** constitute the basic components of the test device of the present invention. If desired, components **1** and **2** may be combined, provided precautions are observed to provide storage stability of the various reagents. Components **4**, **5** and **6** constitute the additional components of the preferred embodiment of this invention which includes an immunochemical test strip for detecting human globin as an indicator of lower intestinal bleeding.

[0087] Sample, for example, a stool sample from a digital rectal examination (DRE), or a water sample taken from around a stool in a toilet bowl, is applied to the sample pad **3**, where any red blood cells are lysed by the detergent impregnated in the sample pad, and developer solution is added. The developer solution may be water, or may include buffer, ethanol and other reagents that assist the reactions and that are compatible with both types of test. From the sample pad, the developer solution mobilizes any hemoglobin released from red blood cells and moves laterally from pad **3** through the flanking regions of the test strip in both directions.

[0088] Heme, if present, mixes with the pseudoperoxidase substrate in substrate region **2** and then mixes with the chromogenic indicator in indicator region **1**, where the color accumulates at the end of region **1**.

[0089] Globin, if present, is detected in the detection region **5** after labeling with the gold-labeled antibody conjugate in substrate region **4**. Excess developer solution and other reagent accumulate in the absorbent pad **6**.

[0090] Clearly, the dual test strip illustrated in FIG. **1** may be encased in a housing adapted for receipt of the sample on pad **3**, with provision (e.g. windows or similar apertures) for visual, or instrumented, detection of the results in indicator region **1** and detection region **5**.

EXAMPLE 1

[0091] Reagent solutions were prepared based on Lam, U.S. Pat. No. 4,071,318, as follows:

Solution A:		
Water	10	mL
Trisodium citrate	213	mg
Citric acid	147	mg
EDTA	6.7	mg
Sodium lauryl sulfate	67	mg
Methyl sulfone	667	mg
Acetone	1.67	mL
Solution B (indicator):		
Tetra methyl benzidine (TMB)	26.7	mg dissolved in
Dimethylsulfoxide	1.67	mL
Solution C (substrate):		
Cumene hydroperoxide	133.3	mg
6-methoxy quinoline	33.3	mg
Triethanolamine borate	667	mg
Solution A	5	mL

[0092] To prepare reactive paper, solutions A, B and C were mixed just before use and added to Whatmans # 1 paper until the paper was soaked. The impregnated reactive paper was hung vertically to drain excess liquid and dried in a warm air current for approximately 30 minutes. The activity of the reactive paper was confirmed by diluting blood obtained from a finger prick in water and adding the dilutions to small pieces of the reactive paper. A 1/100 dilution gave an instant strong blue-green color, 1/1000 produced a strong blue color, 1/10,000 produced a slower developing green color, whereas the 1/100,000 dilution produced a borderline pale blue after 1-2 minutes. Water alone added to the reactive paper produced no color, even when left until dry.

[0093] The reactive paper was tested in a device constructed as shown in FIG. **2**A (FOBT test strip) and **2**B (FOB test strip in housing). FOBT test strips were prepared by laminating the pads A, B, C and D as shown in FIG. **2**A with double sided adhesive (3M #465, 3M MN) to a white plastic (high impact polystyrene)backing (D) and cutting the laminate into test strips approximately 10 mm wide. The test strips were then placed in a waterproof cardboard housing (G) with a port (E) for sample and reagent addition and a window (F).

For ease of reading the test result, the interface between pads B and C was located centrally in the observation window (F) of the test housing (G).

[0094] In the test strips shown in FIG. **2**A:

Pad A:	Non-woven polyester fabric (e.g. Ahlstrom 6613, Ahlstrom, PA) impregnated with 0.1% Triton X-100 detergent.
Pad B:	Reactive paper
Pad C:	White plastic barrier tape.
Pad D	Backing.

[0095] In this test strip, pad A is the sample application pad, and pad B is a combined substrate/indicator region.

[0096] Blood diluted 1/1000 in water was applied to pad A of the test strip of FIG. **2**A via the sample port (E) followed by three drops of water. The liquid migrated from pad A via pad B so that within 25 seconds a strong blue color accumulated at the end of pad B against the white impermeable barrier of pad C. Water alone added to Pad A produced no color in the test window.

EXAMPLE 2

[0097] For long term stability of the FOBT test strip, the substrate and indicator regions were prepared and laminated separately in a test strip constructed as shown in FIG. **3** as a modification of the test strip shown in FIG. **2**A.

[0098] In the test strip of FIG. **3**, pad A is the sample application pad, pad B**1** is the substrate region and pad B**2** is the indicator region. The test strip is prepared as described in Example 1, using substrate paper B**1** and indicator paper B**2** instead of reactive paper B, as follows:

[0099] Substrate paper B**1**: Solution C was prepared, soaked into Whatman's #1 filter paper and drip-dried.

[0100] Indicator paper B**2**: Solutions A and B were mixed, soaked into Whatman's #1 filter paper and drip-dried.

[0101] Both papers B**1** and B**2** were then fully dried in forced air at low heat.

[0102] 10 μL of human blood diluted 1/1000 in water was added to pad A of the test strip of FIG. **3** followed by three drops of a reagent comprised of Bovine serum albumin (3%), Ethanol (10%) and sodium azide in 40 mM sodium borate buffer, pH 8.5. Blue color accumulated at the interface of membrane B**2** and C. No color developed with a water sample alone.

[0103] In an alternative embodiment, the substrate Solution C may be incorporated into pad A during manufacture, and pad B**1** omitted from the test strip.

EXAMPLE 3

[0104] FOBT test strips, as described for Example **1**, were used in combination with commercially available immunochemical (ICT) test strips (InSure FIT, Enterix Inc., N.J.). These ICT test strips are used for detection of human globin as an indicator of lower intestinal bleeding.

[0105] The two test strips (FOBT and ICT) were laid end to end so that the origin of the ICT strip was in contact with pad A of the test strip as described in Example 1. 10 μL of a 1/1000 dilution of human blood in water was added to pad A, followed by four drops of the reagent described in Example 2. The sample migrated in both directions from the point of application and both tests developed a positive result. When water containing diluted blood taken from a beef sample was tested in the same manner it gave a positive result with the FOBT test strip (i.e., positive for hemoglobin), but a negative result with the ICT test (i.e., negative for human globulin). Water alone gave a negative result with both tests.

[0106] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

1. A device for the detection of hemoglobin in a biological sample, comprising a carrier matrix which includes:

(i) a sample application region for receipt of said biological sample;

(ii) a substrate region in liquid-conductive communication with, or combined with, the sample application region and having a pseudoperoxidase substrate applied thereto or impregnated therein, said pseudoperoxidase substrate comprising a peroxide or hydroperoxide reagent; and

(iii) an indicator region in liquid-conductive communication with, or combined with, the substrate region and having an indicator applied thereto or impregnated therein, said indicator producing a detectable response in the presence of heme and said pseudoperoxidase substrate.

2. The device of claim **1**, wherein the sample application region and the substrate region are combined in a combined sample application/substrate region of the carrier matrix.

3. The device of claim **1**, where the sample application region and the substrate region are separate regions of the carrier matrix which are in liquid-conductive communication.

4. The device of claim **1**, wherein the substrate region and the indicator region are combined in a combined substrate/indicator region of the carrier matrix.

5. The device of claim **1**, wherein the substrate region and the indicator region are separate regions of the carrier matrix which are in liquid-conductive communication.

6. The device of claim **1**, wherein the carrier matrix further comprises:

(viii) a second substrate region in liquid-conductive communication with the sample application region and having a detectable antiglobin immunointeractive molecule applied thereto or impregnated therein, said immunointeractive molecule forming a detectable globin-antiglobin complex in the presence of globin; and

(ix) a detection region in liquid-conductive communication with the second substrate region and having an anti-globin immunointeractive molecule immobilized therein, said immobilized immunointeractive molecule immobilizing said detectable globin-antiglobin complex.

7. A method for the detection of hemoglobin in a biological sample, comprising the steps of:

(i) applying said biological sample to a sample application region of a carrier matrix which comprises said sample application region, a substrate region and an indicator region;

(ii) contacting said biological sample with the substrate region wherein said sample is contacted with a pseudoperoxidase substrate comprising a peroxidase or hydroperoxidase reagent; and

(iii) contacting said sample and pseudoperoxidase substrate with the indicator region wherein said sample and substrate are contacted with an indicator which produces a detectable response in the presence of heme and said pseudoperoxidase substrate.

8. The method of claim **7**, wherein the biological sample is contacted with the pseudoperoxidase substrate in a combined sample application/substrate region of the carrier matrix.

9. The method of claim **7**, wherein the biological sample is contacted with the pseudoperoxidase substrate in a separate substrate region of the carrier matrix which is in liquid-conductive communication with the sample application region.

10. The method of claim **9**, wherein the biological sample is contacted with the substrate in the substrate region by permitting or causing the sample to flow from the sample application region to the substrate region of the carrier matrix.

11. The method of claim **7**, wherein the biological sample is contacted with the pseudoperoxidase substrate and indicator in a combined substrate/indicator region of the carrier matrix.

12. The method of claim **7**, wherein the biological sample and substrate are contacted with the indicator in a separate indicator region of the carrier matrix which is in liquid-conductive communication with the substrate region.

13. The method of claim **12**, wherein the biological sample and substrate are contacted with the indicator in the indicator region by permitting or causing the sample and substrate to flow from the substrate region to the indicator region of the carrier matrix.

14. The method of claim **7**, which further comprises the steps of:

(vi) contacting said biological sample with a second substrate region wherein said sample is contacted with a detectable antiglobin immunointeractive molecule to form a detectable globin-antiglobin complex in the presence of globin; and

(vii) contacting said detectable globin-antiglobin complex with a detection region wherein said detectable globin-antiglobin complex is contacted with an immobilized antiglobin immunointeractive molecule to immobilize said detectable globin-antiglobin complex.

15. The method of claim **14**, wherein the second substrate region is in liquid-conductive communication with the sample application region, and the biological sample is permitted or caused to flow from the sample application region to the second substrate region.

16. The method of claim **14**, wherein the detection region is in liquid-conductive communication with the second substrate region, and the detectable globin-antiglobin complex is permitted or caused to flow from the second substrate region to the detection region.

17. The method of claim **7**, wherein said biological sample is a sample derived from the gastrointestinal tract of a patient.

18. The method of claim **17**, wherein the sample is a fecal sample.

19. The method of claim **18**, wherein the sample is a stool sample, or a sample of water containing a stool.

20. The method of claim **17**, wherein the patient is a human.

21. The method of claim **17**, wherein the patient is a livestock animal.

22. The method of claim **21**, wherein the livestock animal is a horse.

* * * * *

Exhibit B

US 20070092401A1

(19) **United States**

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(54) **DEVICES AND METHODS FOR SAMPLE COLLECTION AND ANALYSIS**

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(57) **ABSTRACT**

The present invention provides devices, methods, and kits for the collection of a solid or semi-solid sample and analysis for the presence, absence, or quantity of an analyte. The invention provides an assay device having a housing containing a test element, a results window, and a docking area for receiving and engaging a sample collection slide. The docking area has a sample receiving orifice with one or more fluid transfer structures. In one embodiment the collection slide and device can be used to detect the presence of fecal occult blood (human hemoglobin) in a stool sample. Many other embodiments are described herein.

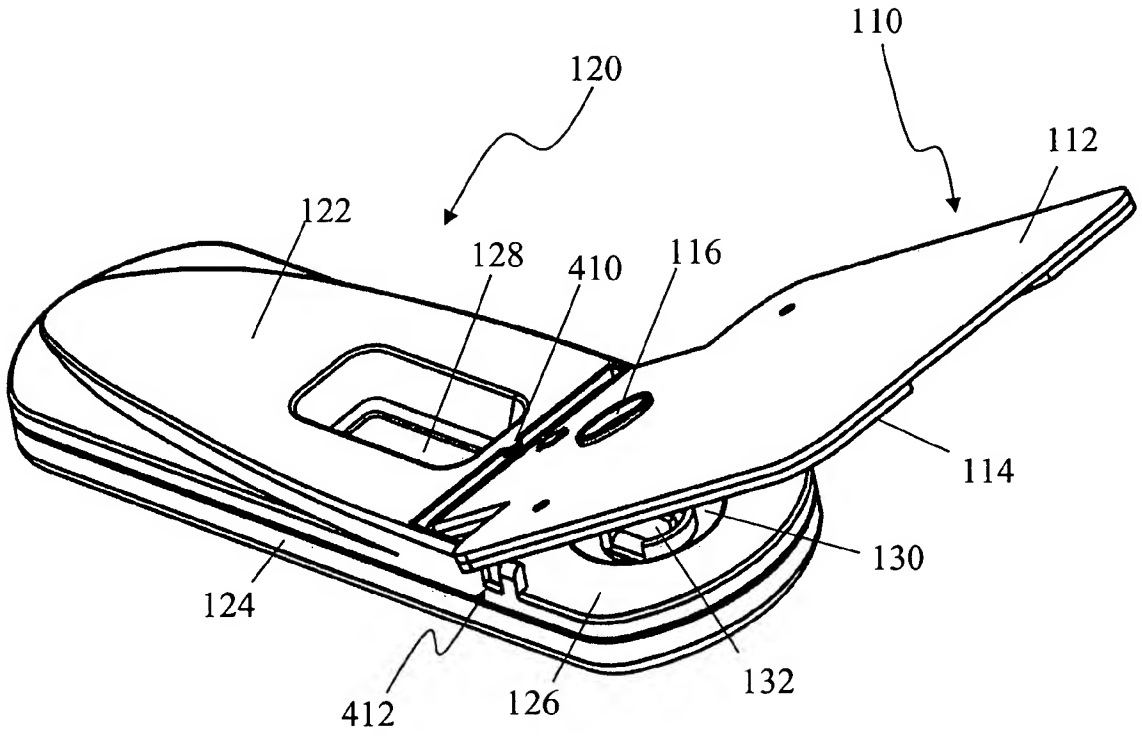

Figure 1

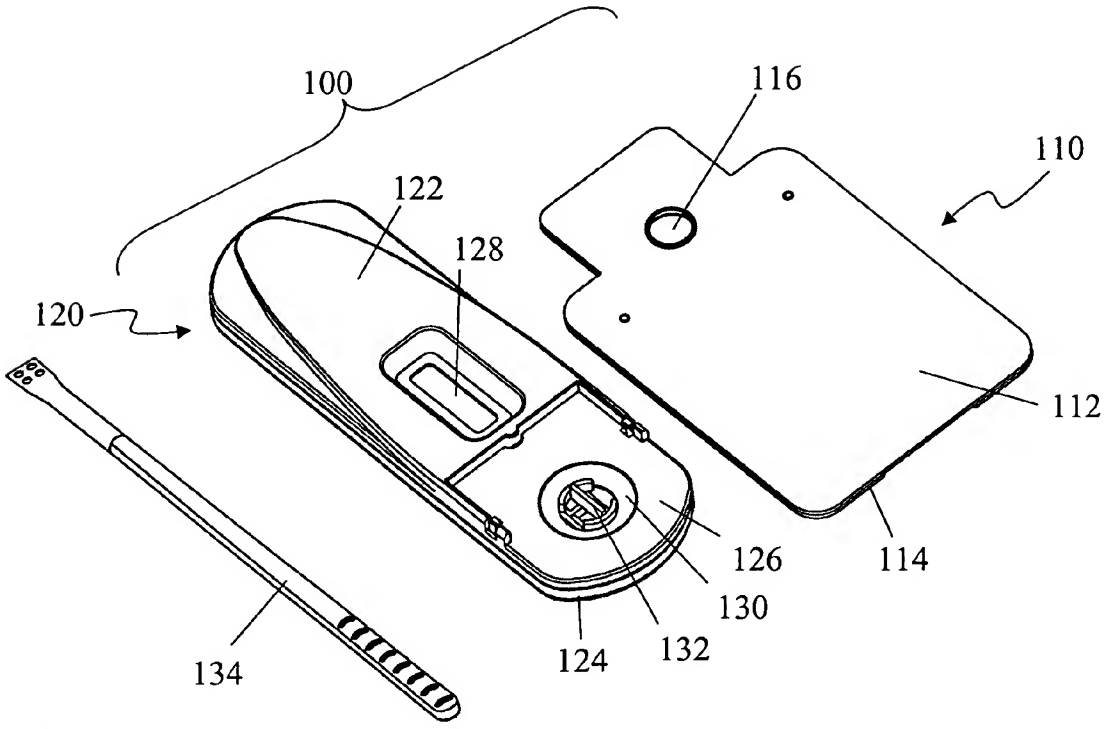

Figure 2

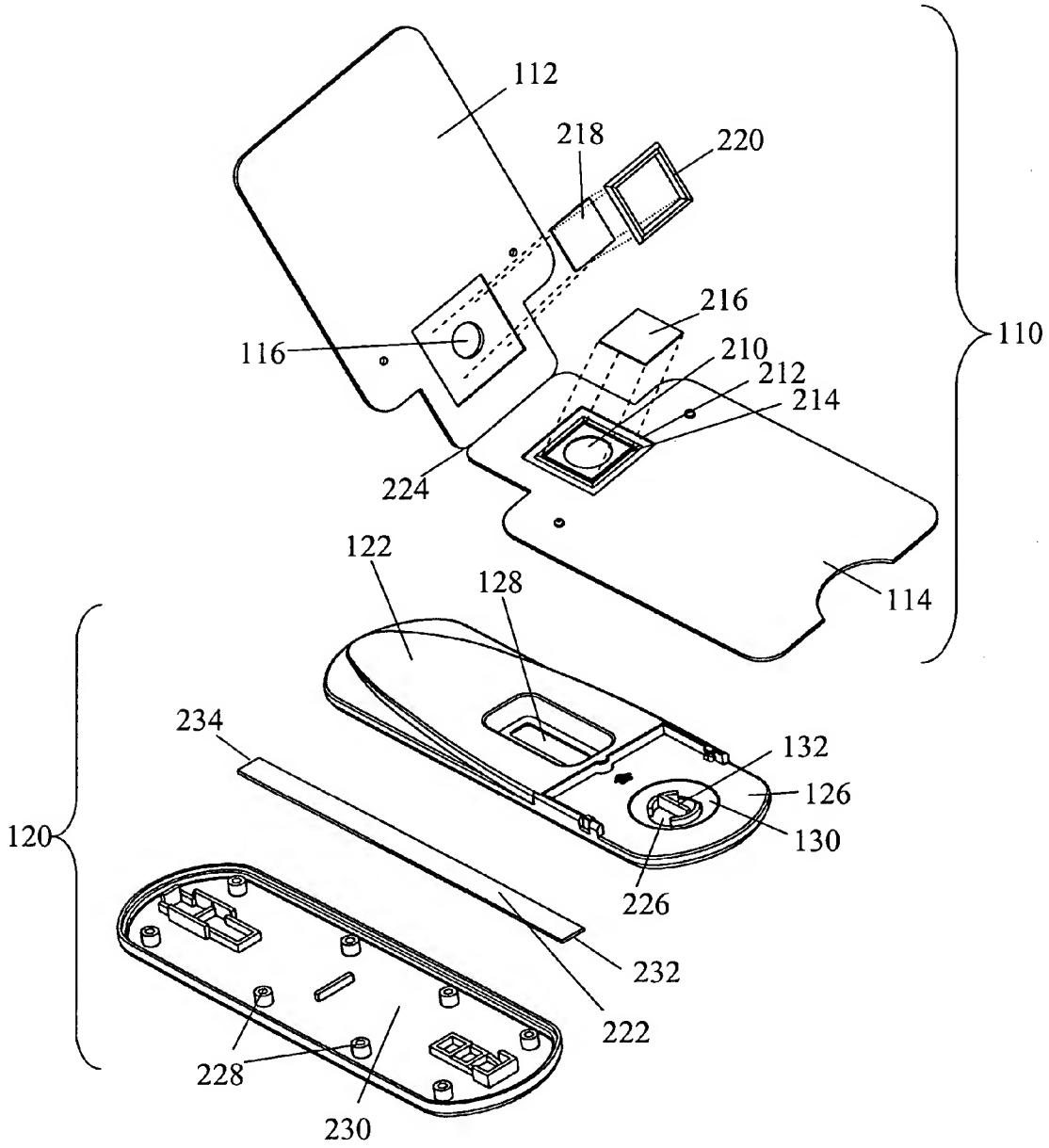
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Figure 3A

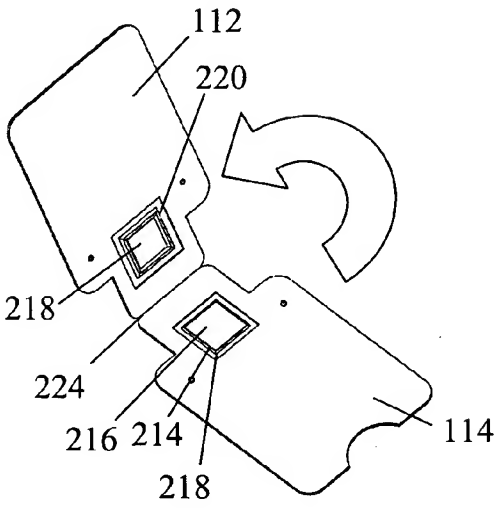


Figure 3B

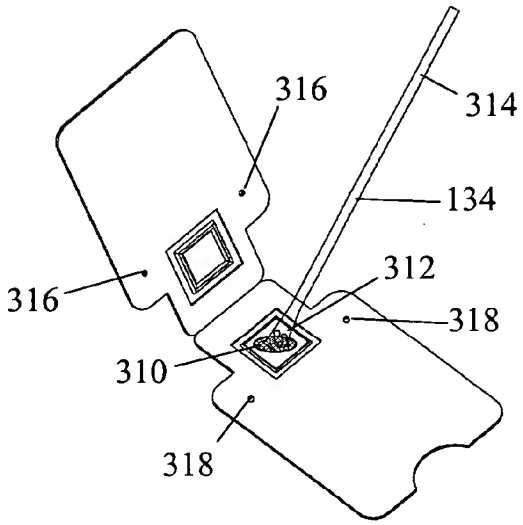


Figure 3C

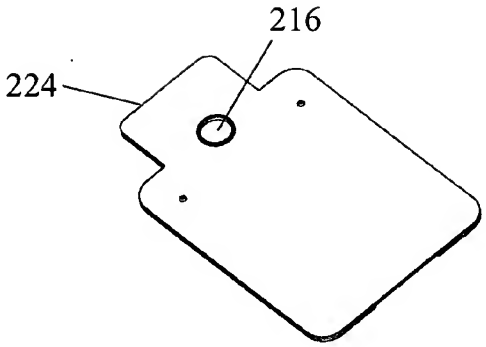


Figure 4

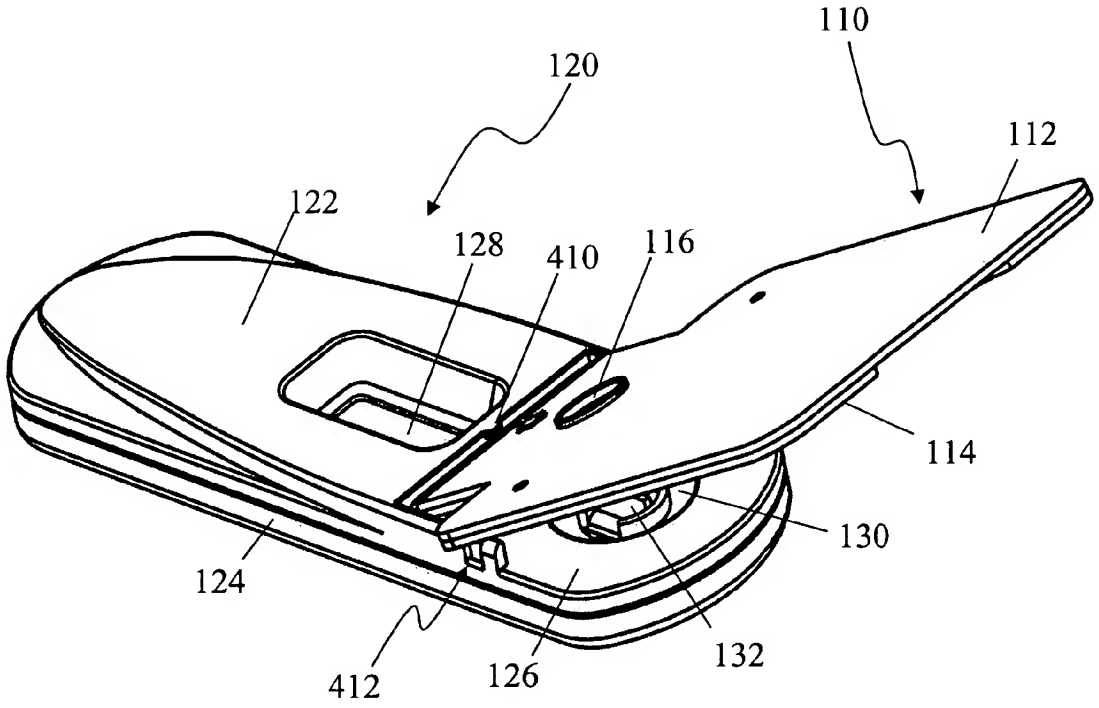


Figure 5

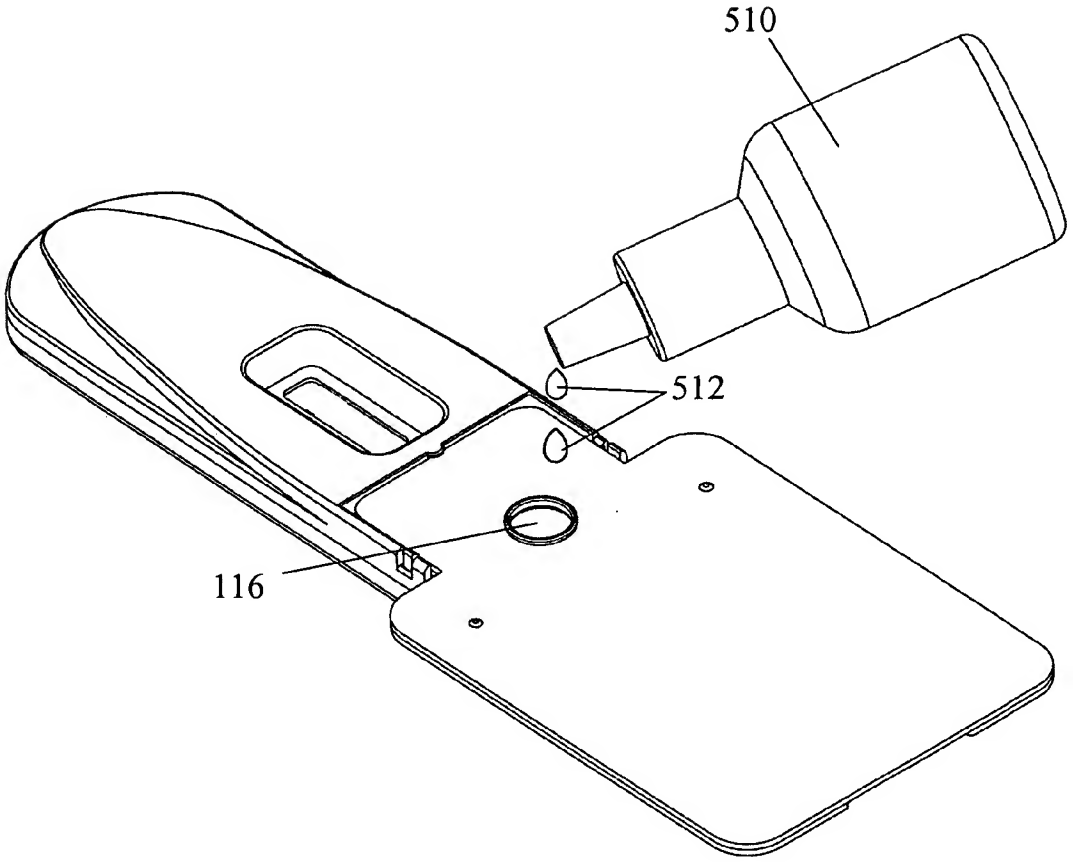
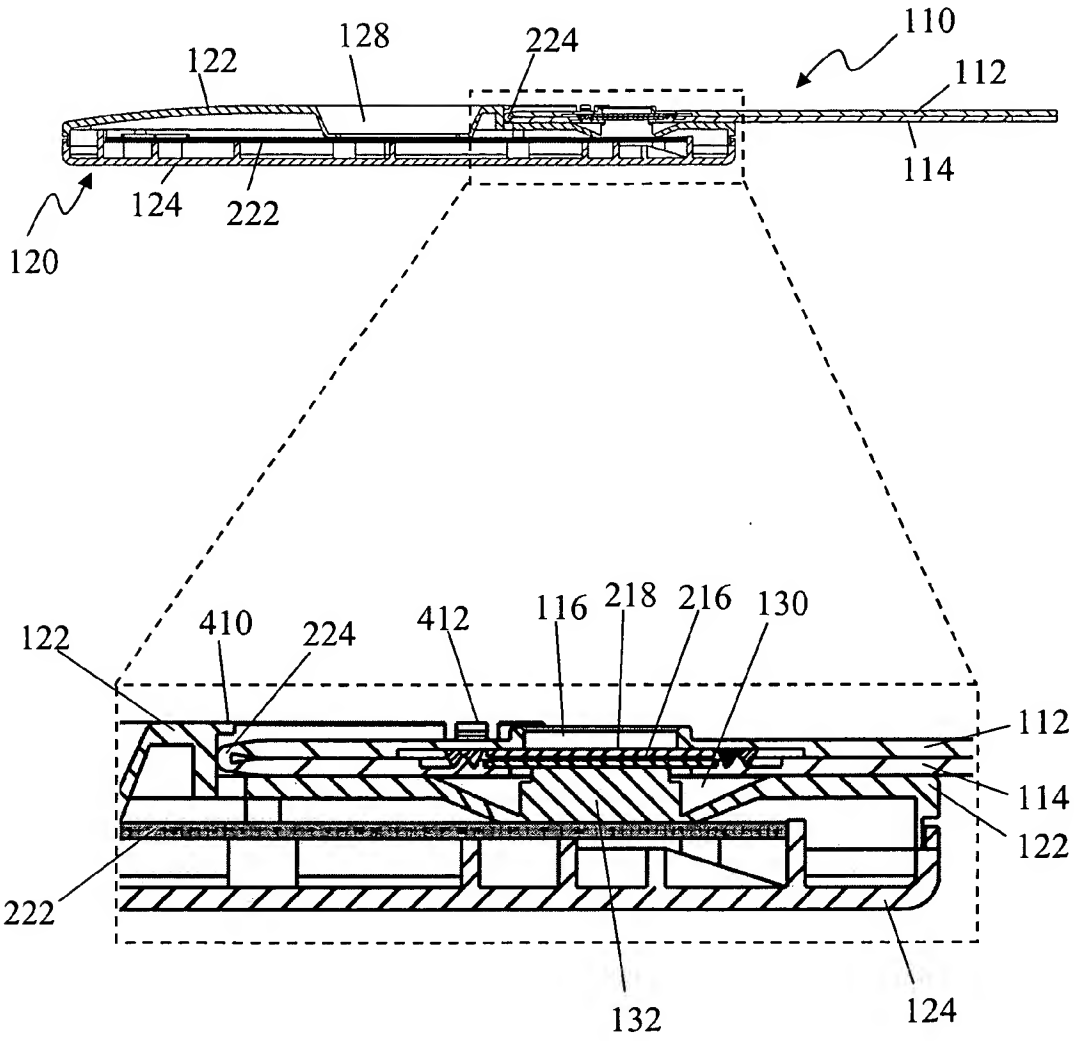


Figure 6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DEVICES AND METHODS FOR SAMPLE COLLECTION AND ANALYSIS

FIELD OF THE INVENTION

[0001] The present invention is directed to devices for the collection of solid or semi-solid biological samples, and their analysis for the presence of analytes.

BACKGROUND OF THE INVENTION

[0002] The following Background of the Invention is intended to aid the reader in understanding the invention and is not admitted to be prior art.

[0003] The detection of occult blood in stool samples is a preliminary method of detecting colon cancer. Traditional methods that detect hemoglobin in a stool sample, such as Guaiac-based chemical methods, are hampered by their inability to distinguish between dietary-derived hemoglobin (i.e. from meat in the diet) and human hemoglobin, which leads to a large number of false-positive test results. To over-come this difficulty, immunoassays specific for human hemoglobin (hHb) have been developed. The antibodies used in these assays are able to distinguish between hemoglobin derived from a human and that from another animal.

[0004] The collection and analysis of occult blood samples presents the problem of the unpleasantness of sample collection and analysis. Presently available devices fail to adequately solve these problems. Therefore, there is a clear and persistent need for a device that reduces the interaction of both the patient and the test operator with the sample while at the same time accurately detecting the presence of hHb in the sample.

SUMMARY OF THE INVENTION

[0005] The present invention provides devices, methods, and kits for collection of a biological sample, and the detection of an analyte in the sample. In one embodiment, the biological sample is a stool sample and the analyte is hemoglobin. The sample is collected on a collection slide that can be used with the device. The device contains a test element, such as a test strip, that has reagents for detecting the analyte. The device also contains a docking area for receiving the collection slide. The docking area contains a sample receiving orifice with one or more fluid transfer structures (e.g., a crossbar extending across the orifice) that facilitate the transfer of fluid from a sample collection card to the device. The fluid transfer structure facilitates the movement of fluid from the card to the device by providing a surface for fluid to adhere to and travel down to the test element.

[0006] In one aspect, the present invention provides a device for detecting an analyte in a sample. The device has a housing that contains a test element, and a docking area for receiving and engaging an external collection slide. The docking area has a sample receiving orifice that has one or more fluid transfer structures within the circumference of the sample receiving orifice. A results window for observing a test result is also provided on the housing.

[0007] In one embodiment, the sample receiving orifice is a well in the housing of the device. In another embodiment, the fluid transfer structure is a crossbar, which can project below, level with, or above the plane of the docking area. The crossbar is in fluid communication with an engaged collection slide. In another embodiment, the docking area has one or more projections for securing the external sample collection slide in position above the sample receiving orifice. The one or more projections can be snap locks. In another embodiment, the docking area is a depression in the housing. The depression can be at least partially circumscribed by a raised area of the housing.

[0008] In another embodiment, the test element is made of a bibulous matrix, which has a sample application zone (in fluid communication with the one or more fluid transfer structures), a reagent zone (containing reagents for conducting an assay) and a detection zone. The detection zone contains a test line, for visually detecting the presence or absence of the analyte at the test line. The test line can also contain a specific binding molecule, for the analyte, immobilized on the matrix. In some embodiments, the specific binding molecule is an antibody. In other embodiments, the specific binding molecule on the test line binds to human hemoglobin. In still another embodiment, the reagent zone contains labeled specific binding molecule for the analyte.

[0009] In another aspect, the present invention provides methods of detecting the presence or absence of an analyte in a sample contained in a sample collection slide. The methods involve placing a collection slide containing the sample into a docking area of a device for detecting analyte in the sample as described herein. In one embodiment, the collection slide has a first water resistant card with an eluent orifice, a second water resistant card hingeably connected to the first card and having a solvent orifice. The collection slide can have both an open position and a closed position, and a sample collection surface is present between the solvent and eluent orifices, when the collection slide is in the closed position. The method further involves applying an extraction buffer to the solvent orifice of the collection slide, allowing the extraction buffer to pass through the sample area and through the sample receiving orifice and test element, and observing a test result in the results window.

[0010] In one embodiment, the test element is a bibulous matrix having a sample application zone in fluid communication with the one or more fluid transfer structures, a reagent zone, including reagents for conducting an assay, and a detection zone having a test line for detecting the presence or absence of the analyte. The test line can also include specific binding molecules for the analyte. In another embodiment, the test line contains reagents for conducting a chemical test.

[0011] In another aspect, the present invention provides a kit for collecting a biological sample. The kit includes a test device of the present invention, a collection card and a sample collector, as described herein, provided in a package. In a further embodiment, the kit includes one or more bottles containing buffers. The buffers are for conducing an assay according to the instructions for use.

[0012] The summary of the invention described above is not limiting and other features and advantages of the invention will be apparent from the following detailed description, as well as from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 provides a perspective view of an embodiment of the invention, which includes a sample collection

slide **110** and a test device **120** that engages the collection slide. Also shown is the sample collector **134** for applying the sample to the collection slide.

[0014] FIG. **2** provides an exploded view of the devices shown in FIG. **1**.

[0015] FIGS. **3**A-**3**C illustrate application of a sample to the collection slide. FIG. **3**A illustrates an opened collection slide, showing a cover pad **218** and a collection pad **216**. FIG. **3**B illustrates application of the sample **310** to the collection pad. FIG. **3**C illustrates a closed collection slide.

[0016] FIG. **4** illustrates a collection slide **110** engaging the docking area **126** of a test device.

[0017] FIG. **5** illustrates application of extraction buffer **512** to the solvent orifice **116** of an engaged collection slide.

[0018] FIG. **6** provides a cross-sectional view of a collection slide **110** engaged in a test device **120**.

DETAILED DESCRIPTION

[0019] In the following detailed description, reference is made to the accompanying drawings that form a part hereof, and in which is shown by way of illustration specific embodiments in which the invention may be practiced. It is understood that with reference to the present disclosure other embodiments may be utilized and structural changes may be made without departing from the scope of the present invention.

Collection Slide

[0020] The present invention provides collection slides for collecting a solid or semi-solid sample. In some embodiments the sample is a biological sample, such as a stool sample. The present invention also provides devices for detecting the presence of analytes in the sample, and methods for collecting the sample.

[0021] The test device of the present invention can be used with an external collection slide **110**. With reference to FIGS. **1**-**5**, the collection slide **110** has a first card **114** and a second card **112**. The first and second cards may be made of any appropriate material. For example, the cards can be made of a resilient, water resistant or water-impermeable material, such as plastic, coated cardboard, metal or glass. In one example, the cards are hingeably connected to each other, for example by a hinge **224** (FIG. **2**). By "hingeably connected" is meant that the two cards are connected to each other at their first ends and have free ends movable towards and away from each other by movement about the hinge. A wide variety of hinge connections may be advantageously used. In the example shown in the figures, the collection slide is manufactured of injection molded plastic and the two cards are connected by a living hinge, as depicted in FIG. **2**. In other examples, the hinge can be one or more flaps of material that bind the two cards together and allow for one card to be folded onto the other card. In another example the cards are present as separate cards that can be secured together, for example by a locking mechanism. The second card has a buffer or solvent orifice **116**, through which an extraction buffer **510**, **512** can be applied to a collected sample (FIGS. **1** and **5**).

[0022] The collection slide has an open position and a closed position (compare FIGS. **1** and **2**). As illustrated in FIG. **2**, the first card has an eluent orifice **210** and the second card has a solvent orifice **116**. The solvent and eluent orifices are positioned on the cards so that when the collection slide is in the closed position, the two orifices are in alignment. By the orifices being "aligned" or "in alignment" is meant that a liquid applied to the solvent orifice in the second (or top) card in sufficient quantity will pass through the sample collection area and through the eluent orifice.

[0023] Referring to FIG. **2**, a cover pad **218** is present on the inner surface of the second card and overlaying the buffer orifice **116**. The cover pad and sample collection pad can be made of any suitable material that retains sample and allows the passage of fluid. Examples of materials suitable for the cover pad and/or sample collection pad are polyester mesh, fibrous or bibulous materials, paper or paper-based materials, synthetic fabrics, meshes and wools, coated or supported papers, polyesters, nylon membranes, nitrocellulose, glass wool, treated paper, absorbent paper, or a material made of a cellulose base. In the example shown, the cover pad **218** is circumscribed by a gasket **220**. With reference to the present disclosure the person of ordinary skill in the art will realize many other materials suitable for the cover pad and/or sample application pad.

[0024] On the first card is present an eluent orifice **210**, which is overlaid with a sample collection pad **216**. The sample collection pad **216** can be made of any suitable material that retains sample and allows for the passage of fluid. In various examples the sample collection pad **216** is made of the same types of materials as the cover pad. The sample collection pad can be circumscribed by ridge **214** and groove **212**, or by a series of ridges and grooves. The cover pad and the collection pad can be made of any suitable material that retains sample and allows for the passage of fluid. Examples are provided above with respect to material for the cover pad. The material should also have sufficient resiliency to withstand the mechanical pressure of the sample application. Preferably, the material does not deteriorate or tear when wet.

[0025] Common difficulties with stool sample collection include that patients tend to over-apply sample to collection slides, which can cause interference when the assay is an immunoassay. Collection slides used with a test device of the present invention can desirably limit the amount of sample that can be applied to the slide while requiring no direct sample manipulation by the technician conducting the test. Using such a slide, the amount of sample collected is limited to the sample collection area since the cover pad and sample collection pad are circumscribed by the sealing structures (e.g., a gasket and groove) when the slide is in the closed position. When the collection slide is moved to the closed position, the interaction of the sealing structures (e.g., the interaction of the gasket with the groove and ridge) separates the sample within the sample application area from sample applied outside the sample area. After the sample has been applied to the sample collection area, the collection slide is closed and retained in a locked position, thereby limiting the volume of sample retained within the sample area, because excess sample is squeezed out as the two cards are pressed together. The sealing structures can also be structures other than a gasket, ridge, and groove. For example, the structures can be a pressure sensitive adhesive or a wax bead (or beads) present on or around the sample collection pad and/or cover pad, which seal the sample

collection pad when the two cards are closed and pressed together. The "seal" does not have to be a tight seal, just that it generally impedes the passage of sample into or out of the sample collection area when the collection slide is in the closed position. With reference to this disclosure the person of ordinary skill will realize many other structures that will find use in other examples of the collection card.

[0026] The cover pad and/or collection pad can be treated with reagents that improve the flow of aqueous liquids through them. Additionally, these treatments also improve the elution of the analyte of interest from the dried sample within the sample area. In one example the pads are treated with surfactants to inhibit proteins from sticking to the pads and to promote protein solubilization. A wide variety of commonly used anionic and non-ionic surfactants may be advantageously used in various concentrations. Some cationic and amphoteric surfactants may also find use in the present invention. Some examples of surfactants that may be used to treat the pads include, but are not limited to, the polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (e.g., the BRIJ® (ICI US, Inc.) series of surfactants). Other useful surfactants include octyl phenol ethoxylate surfactants (e.g., polyethyrene glycol mono-p-iso-octylphenyl ether and other Triton® (Rohm & Haas, Philadelphia, Pa.) series surfactants), polyoxyethylene derivatives of sorbitan esters (e.g., the Tween® (ICI Americas, Inc.) series of surfactants) and block copolymers based on ethylene oxide and propylene oxide and represented by $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$ (e.g., the Pluronic® (BASF) series of surfactants). With reference to the present disclosure, a surfactant can be conveniently chosen using known surfactant selection techniques, such as by using a commercially available surfactant tool kit, for example, the Reagent Developer's Surfactant Took Kit (Pragmatics, Inc., Elkhart, Ind.), or a similar kit. These kits provide a convenient method of testing a large number of surfactants on a specific application, in order to optimize protein extraction and flow-through.

[0027] In some embodiments the pads are treated with a buffer containing a component that improves analyte stability. Buffers can also condition the sample to promote optimal binding between the analyte and the specific binding reagents (e.g., antibodies or antibody fragments), which can be utilized in the assay. This can be performed, for example, by adjusting the pH of the analyte. Buffers having these useful qualities include, but are not limited to, Tris(hydroxymethyl)aminomethane buffer, phosphate buffer, borate buffer, tartrate buffer and phthalate buffer.

[0028] The cover pad and/or sample application pad can also be treated with one or more polymers, which can also have the property of improving analyte stability and elution. Polymers sometimes used in protein purification can be useful for this purpose. Examples of useful polymers include, but are not limited to, polyvinylpyrrolidone (PVP), poly(methylvinylether-co-maleic anhydride, polyethylene oxide (PEO), polyelthylene glycol (PEG), copolymers of methyl vinyl ether and maleic anhydride (e.g., poly(methylvinylether-co-maleic anhydride), polyvinylalcohol (PVA), vinylpyrrolidone/vinylacetate, bony fish gelatin (from fish of the class Osteichthyes), crosslinked polyacrylic acid polymer, hydroxypropylcellulose (HPC), sodium carboxymethylcelluose (CMC), sodium polystyrenesulfonate, sodium carageenin, acrylic latex, and hydroxyethylcellulose (HEC)). These polymers are commercially available (e.g., from Pragmatics, Inc., Elkhart, Ind.), and are conveniently formulated in a polymer tool kit. They can therefore be used systematically to determine the advantages of particular polymers in particular applications.

[0029] To improve analyte extraction, the pads may also be treated with a non-specific protein, which functions as a blocking agent. Any protein may be used for this purpose including, but are not limited to, bovine serum albumin, egg white albumin, and casein.

[0030] The cover pad and sample application pad can also be treated with a preservative to increase the shelf-life of the collection slide. A "preservative" is a naturally or synthetically produced chemical added to inhibit microbial growth or undesirable chemical changes. Any preservative can be used that provides the preserving effect and does not interfere with the assay. Examples of useful preservatives include, but are not limited to, 5-chloro-2-methyl-isothiazol-3-one (e.g. ProClin® 300 (Supelco, Inc., Bellefonte, Pa.) and sodium azide. With reference to the present disclosure the person of ordinary skill will realize many other preservatives that will find use in the present invention.

[0031] The cover pad and collection pad form the top and bottom walls of the sample collection area, and serve to eliminate excess sample from the sample collection area. When the structures on the cards are a gasket, ridge, or groove, they can also be situated on the opposite cards as those described above.

[0032] In certain sample collection slides, one of the cards of the collection slide is provided with structures for securing the first and second cards in a closed position. In one example short pins **316** (FIG. **3**B) are present on the interior surface of one card. The opposite card is provided with holes **318** that mate with the pins. When the collection slide is closed, the pins are inserted into the holes and lodged with sufficient resistance to hold the collection slide in a closed or "locked" position. In one example this action may advantageously cause a snapping noise, alerting the patient that the collection slide has been properly closed. Other methods of securing the collection slide in a closed position can also be incorporated into the slide. For example, a clip that fits over the outside of the two cards and holds them together could be used in one example, or snaps present on the inner surfaces of the two cards can be used in another example. With reference to the present disclosure the person of ordinary skill will realize other structures for retaining the collection slide in the closed position.

Sample Collector

[0033] The present invention also provides a sample collector **134**, such as the embodiment shown in FIG. **1**. The sample collector has a handle **314** (FIG. **3**B) and a spatula **312** for moving the sample. In one embodiment the spatula is perforated with a plurality of holes, which reduces the liquid content of the sample, and also serves to reduce application of excess sample to the sample collection pad. In various embodiments the spatula portion of the device is perforated with 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more holes. The spatula portion of the collector can be generally flat, or can have a concave or curved (spoon-like) shape. This device can be made of any suitable material (e.g., plastic). In one embodiment, the spatula portion of the device is made

of a soft plastic, and the handle is made of a harder plastic. This will enable the spatula to bend when sample is applied to the sample collection pad and lay on the pad. The perforations in the spatula portion will also act as an aid in applying an even sample to the pad.

Methods of Collection

[0034] In another aspect the present invention provides methods of collecting a sample. In one embodiment the sample is a stool sample. One embodiment of the method of sample collection and operation of the collection slide and assay device is illustrated in FIGS. **3**A-**3**C. Referring to FIG. **3**A, the patient opens the collection slide to expose the inner surfaces of the first and second slides, revealing the cover pad and sample collection pad. A small amount of stool sample is applied to the sample collection pad **216**. The collection slide is then closed (FIG. **3**C). The present collection slide eliminates excess sample by providing a sample collection area, with a design such that only sample in the sample collection area will be incorporated into the assay. When the collection slide is closed, a structure the first card engages a structure second card, forming a wall that circumscribes the sample collection area. In one embodiment the structure on one card is a gasket, and the structure on the opposite card is a groove and a ridge. When the collection slide is in the closed position, the solvent or buffer orifice, the sample area, and the eluent orifice are all vertically aligned. In this position, when buffer is applied to the buffer orifice, it flows through the cover pad and into the sample collection area, and then out of the eluent orifice, thereby rinsing the sample in the process and solubilizing analyte of interest contained in the sample. Additionally, the buffer dilutes the sample and conditions it for optimal binding of analyte by the specific binding reagents on the test element. After passing through the eluent orifice, the liquefied sample is then passed along the fluid transfer structures of the device and through the sample receiving orifice, and to the test element of the device.

[0035] Human hemoglobin breaks down rapidly when left in a wet sample. To prevent analyte degradation, the methods can incorporate the step of drying the sample. This step can involve leaving the collection card exposed to air for a certain period of time to allow it to air dry, or drying the sample in an oven at 45° C. The step can also involve placing the closed collection slide into a container containing desiccant. The container can be a sealable pouch (e.g., a mailing pouch). After drying (or placing the collection slide in a sealable pouch containing a desiccant), the collection slide can be presented to a health care facility for analysis.

Assay Device

[0036] The present invention provides devices for detecting the presence of analytes in the sample, and methods for collecting the sample. The devices of the present invention can be used with collection slides for collecting a solid or semi-solid sample. In some embodiments of the present invention, the sample is a biological sample, such as a stool sample.

[0037] Referring to FIGS. **1** and **2**, the assay device of this embodiment has a housing consisting of a top portion **122** and a bottom portion **124**, which engage one another and lock together. The housing may be constructed of any suitable material such as, for example, plastics, pressed hardboard, metals, ceramics, polymers (e.g., polycarbonate, polypropylene, cycloolefins), and other materials. In the embodiment illustrated in the Figures, the housing is made of molded plastic. The top and bottom portions can engage one another by any convenient means, such as parts that snap together, glue, micro-welding, and other means. In the embodiment illustrated in FIG. **2**, the top portion has a series of pins on the inner surface (not shown) which snap-fit snuggly into a corresponding series of raised rings **228** on the inner surface **230** of the bottom portion, thereby securing the top and bottom portions of the assay device in a locked position.

[0038] A docking area **126** for receiving and engaging a collection slide is located on the assay device. The collection slide may be "loaded" meaning that it contains a sample to be analyzed. The docking area may be of any shape, and can mate with a portion of the collection slide carrying the sample collection area. In one embodiment the docking area can receive and engage an external collection slide. An external collection slide is one that can be loaded separately from the assay device, and is not physically connected to the device at the time of sample loading. By "receiving and engaging" a collection slide is meant that the assay device and collection slide are placed into the "test position." The "test position" is when the sample application pad and the fluid transfer structure(s) **132** are in liquid communication.

[0039] The docking area can also receive the collection slide in reversible fashion, meaning that the collection slide can be removed from the device after buffers are applied and sample eluted from the collection slide. As illustrated in FIG. **4**, in this embodiment the collection slide is snapped into the docking area by fitting the hinged edge of the collection slide under a tang **241** (also see FIG. **2**). The collection slide is then pressed down onto the docking area and snapped into a locked position under one or more projections **240**. The projections hold the collection slide flush with the docking area. In other embodiments the collection slide is placed into the docking area of the assay device. In one embodiment the docking area can have a part that fits over the collection slide to hold it in place (e.g., an overhang that grips an end of the collection slide). When in place, the sample collection pad and the fluid transfer structure(s) are placed into fluid communication. The buffer orifice is exposed to receive buffer, and buffer applied to the buffer orifice passes through the sample collection pad and to the fluid transfer structure(s). In one embodiment the docking area is configured to receive the collection slide against an exterior surface of the assay device, so that the sample collection area and fluid transfer structure(s) are brought into liquid communication. The docking area can have projections for holding the collection slide securing in the test position.

[0040] In other embodiments the docking area can receive the collection slide into the interior of the device. For example, the collection slide can be slid into an opening in the housing of the device so that the sample application pad is placed into liquid communication with the fluid transfer structures. In another embodiment the sample transfer orifice is the only orifice in the assay device for receiving sample or assay fluids, and the sample and assay fluids both enter the device through the sample transfer orifice. "Assay fluids" refers to buffers or other reagents utilized during the

assay. Thus, in these embodiments the sample transfer orifice is the sole orifice for receiving sample and fluids into the device.

[0041] As illustrated in FIG. **1**, in one embodiment the docking area contains an indentation or well **130** having one or more fluid transfer structures **132** disposed therein. The fluid transfer structures can take any form that projects toward the collection slide and touches or nearly touches the exterior surface of the sample collection pad. For example, the fluid transfer structure(s) could be one or more raised bars attached to edge of the well. In another example, the fluid transfer structures can be a number of projections that extend towards the sample application pad of the collection slide. Any suitable number of projections can be used, such as one or 2 or 4 or 6 or 8 or 10 or 12, or 2-6 or 2-8 or 2-10 or 2-12 or 4-8.

[0042] In the embodiments shown in FIG. **2**, the fluid transfer structure is present in the top portion of the housing, in the sample transfer orifice **226** and lies beneath, flush with, or slightly protruding through the plane of the docking area. In various examples, the fluid transfer structure can protrude 1 mm or 2 mm or 3 mm or 4 mm or 5 mm, or any suitable distance. The "plane" of the docking area is that spatial plane extending over the surface of the docking area and over the well.

[0043] In the embodiment illustrated, the fluid transfer structure is a cross bar spanning the diameter of the sample receiving orifice. In the case of more than one bar, the bars can be arranged parallel or at an angle to each other and intersecting (e.g., to form an "X" shape, or a grid, square, triangle, or a honeycomb pattern). In other embodiments the bars can connect any two points on the circumference of the sample receiving orifice. In other embodiments one or more vertically projecting prongs situated inside the well can also be used as fluid transfer structures. A straight or curved wall of any shape can be adapted to use as a fluid transfer structure.

[0044] The fluid transfer structure facilitates the transfer of eluate emerging from the bottom of the sample collection pad to the test element. In one embodiment, when the collection slide is snapped into the docking area, the buffer orifice, cover pad, sample collection pad, eluent orifice, and fluid transfer structure(s) are all generally in vertical alignment with each other (FIG. **6**). In this embodiment the fluid transfer structure projects towards, into, level with, or above the plane of the docking area, so that the fluid transfer structure and the outer surface of the sample collection pad are placed into fluid communication through the eluent orifice. By being in "fluid or liquid communication" is meant that fluid passing through the sample collection area and through the sample collection pad is passed to the fluid transfer structure. The sample collection pad and fluid transfer structure may make direct physical contact or be slightly apart from one another, but are retained in fluid communication.

[0045] Cohesion refers to the attraction of one water molecule to another resulting from hydrogen bonding. Adhesion is similar to cohesion except that adhesion involves the attraction of a water molecule to a non-water molecule, such as a surface. The fluid transfer structures in the present invention facilitate the movement of eluate collected on the exterior surface of the sample transfer pad by providing a surface for water molecules to be attracted to by adhesion. When the fluid transfer structure breaks the surface tension of the eluate, the eluate adheres to the surface of the fluid transfer structure. Adhesion of the eluate to the fluid transfer structure, in combination with the weight of the eluate, moves the eluate toward the sample pad of the test strip. Thus, the eluate flows to the test strip using adhesive forces along the fluid transfer structure(s). When the eluate contacts the sample pad of the test strip, the eluate is drawn into the sample pad by capillary action. Sufficient elution buffer is applied to the test card buffer orifice, that enough eluate is produced to flow, by capillary action, to the end of the test strip opposite the sample pad, and the test performed on the test strip can function properly.

[0046] As illustrated in FIGS. **2** and **6**, a test element **222** is provided with the housing, and in this embodiment is contained within the housing. In this embodiment the test element is permanently situated within the housing of the device, meaning that it is not removable from the housing or inserted during the assay, but is an integral part of the assay device. Referring to FIG. **6**, the fluid transfer structure is in fluid communication with the test element. In one embodiment, the test element is a bibulous test strip suitable for performing a lateral flow assay. A variety of test strips are suitable for use in the assay device. In one embodiment the test strips consist of a bibulous matrix, for example nitrocellulose, and/or other suitable materials. The matrix can have a sample loading zone, a reagent or label zone, and a detection zone. A variety of other test strips will also find use in the present invention. In some embodiments a sample loading zone is present at one end of the test strip for the application of sample to the test strip. The sample loading zone is the portion of the test strip in liquid communication with the transfer material. Reagents for conducting the assay or conditioning the sample can also be present at the sample loading zone, or they can be present in a separate reagent or label zone. These reagents can serve a variety of purposes, for example preparing the sample for optimal binding with a specific binding molecule, or improving the stability of an analyte of interest. By "conditioning" a sample is meant adjusting the characteristics of the sample to promote or improve the reaction that detects the presence of the analyte. For example, buffers may be included to adjust the pH of the sample. If the sample contains substances that compete for binding with a specific binding molecule used in the assay, a secondary blocking antibody can be included to bind the substance, or if enzymes that would degrade the specific binding molecules for the analyte are present in the sample, one or more enzyme inhibitors can be added to the reagent zone.

[0047] The sample loading zone is present at the upstream end **232** of the test strip. Towards the downstream end of the test strip **234** is the reagent zone, which is followed by a detection zone. The reagent zone can include reagents for conditioning the sample, reagents for labeling the analyte (e.g., specific binding molecules if the assay is a sandwich format immunoassay) or labeled analyte analogs (e.g., if the assay is a competitive format immunoassay). In some embodiments the reagent zone contains a labeled specific binding molecule for the analyte present on the matrix in a dried form, and which can be solubilized by sample fluid as it passes along the matrix. In one embodiment the specific binding molecule is an antibody or fragment thereof. In one embodiment the analyte is human hemoglobin (hHb), and

the labeled specific binding molecule is an antibody that binds hHb. The antibody can be labeled by any suitable methods, for example, a metal sol, colored latex beads, and dyes. In some embodiments the sample loading zone and the reagent zone over-lap. In other embodiments there are present a series of reagent zones located on the test strip.

[0048] A "specific binding molecule" refers to a molecule that binds to a target analyte (e.g., human hemoglobin) and does not substantially bind to any other molecule present in the sample. In some embodiments a specific binding molecule can also bind to a molecule that correlates with or indicates the presence of an analyte of interest in a sample. By substantial binding is meant that binding occurs to an extent that will affect the result of an assay performed with the specific binding molecules, i.e., a less optimal or less accurate result will be obtained. A small amount of non-specific binding that may occur and that does not change the result of the assay is not considered substantial binding. In some embodiments the specific binding molecule can be an antibody or an antibody fragment (e.g., the Fab region of an antibody), an antigen, a receptor or fragment of a receptor that binds a ligand, or a member of a biotin-streptavidin pair or other type of binding pair.

[0049] The detection zone is the area of the test strip where the presence of the analyte is detected. In some embodiments the detection zone contains a test line for visually detecting the presence or absence of the analyte of interest at the test line. The test line can be of any shape, and need not be only a line. The test line can have a specific binding molecule for the analyte. When human hemoglobin is the analyte of interest, the specific binding molecule on the test line binds to hHb. In this embodiment the specific binding molecule binds to human Hb, and does not bind to hemoglobin that might be present from the diet, in order to avoid false positive results.

Methods of Detection

[0050] Another aspect of the present invention provides methods of detecting the presence or absence of an analyte in a sample using the assay device of the present invention. In one embodiment of the present method, a collection slide containing the sample is placed into the docking area of an assay device, as shown in FIG. **4**. Extraction buffer **512** is applied to the buffer or solvent orifice of the collection slide. The extraction buffer elutes the analyte of interest from the sample, if the analyte is present. Buffer applied to the buffer orifice flows through the cover pad and into the sample collection area containing the dried sample. The dried sample is rehydrated and a portion of the sample elutes out of the collection slide, through the eluent orifice. In one embodiment the buffer is pulled through the collection pad and onto the fluid transfer structure(s) by the fluid transfer structure(s) breaking the surface tension of the eluate. Excess buffer eluted from the collection slide is collected in the well surrounding the fluid transfer structure(s). Eluate on the fluid transfer structure(s) flows by gravity and capillary action into the application zone of the test strip, and then (by capillary action) to the downstream end of the test strip. As the eluate flows from the transfer structure(s) into the test strip, excess eluate held in the well may be transferred to the test strip, by the transfer structure(s).

[0051] As the eluate flows through the sample loading zone and reagent zone of the test strip, it dissolves reagents for conducting the assay present in the loading zone or reagent zone. In one embodiment these reagents are dried on the test strip. Reagents can also be included that condition the eluate for optimal detection, as described above. For example, if the assay is a sandwich format immunoassay reagents may include specific labeled binding molecules for the analyte, such as an antibody or fragment thereof. In one embodiment the specific binding molecule is a gold-labeled anti-hHb antibody or antibody fragment. If the analyte is present in the sample, the labeled specific binding molecule would capture the analyte and form a labeled, soluble complex, which is detected in the detection zone. The eluate continues to flow through the test strip to the detection zone, which contains a test line having specific binding molecules for the analyte. For example, the specific binding molecule can be an unlabeled antibody against the analyte, which binds at an epitope different from that of the labeling reagent. If the assay is a sandwich assay, the specific binding molecule in the test line captures the labeled antibody-analyte complex, and forms a visually detectable line indicating that the analyte is present in the sample. The test result therefore appears in the results window **128** located in the top portion of the housing.

[0052] In another embodiment the assay is a competitive format immunoassay. In this embodiment, the label zone or reagent zone of the test strip contains a labeled analog of the analyte, such as a gold-labeled hHb analog. If no analyte is present in the sample, the labeled analyte analog binds the antibody on the test line. Therefore a positive result on the test line indicates that no analyte is present in the sample. When analyte is present, it competes with the labeled analog to bind the antibody on the test line. As the concentration of analyte in the sample increases, the amount of analog that binds to the test line decreases. Therefore, a lighter line or no line indicates the presence of analyte in the sample.

[0053] A procedural control can also be included in the detection zone. The procedural control can be present as a line, and will always appear whether or not analyte is present in the sample. Absence of a positive result from the procedural control indicates an invalid assay.

[0054] In other embodiments the eluate is tested by means other than an immunoassay. For example, the analyte-containing eluate could be detecting using a chemical means, such as a Guaiac test or other chemical means.

Types of Samples and Analytes

[0055] A "sample" is any material to be tested for the presence, absence, or quantity of an analyte. In one embodiment the sample is a biological sample, such as a stool sample. But any type of sample can be assayed using the present invention, as long as it contains an analyte to be detected that can be solubilized and can be passed through the collection slide and into the assay device. The sample can be in many forms, such as solid, semi-solid or highly viscous materials, such as stool, soils, tissues, blood, bodily fluids, or macerated organs. The sample may also be an oral or vaginal swab.

[0056] A variety of analytes may be tested for using the present device. Examples of analytes that can be detected using the present invention include, but are not limited to, hemoglobin or other blood components, creatinine, bilirubin, nitrite, protein (nonspecific), hormones (e.g. human

chorionic gonadotropin, luteinizing hormone, follicle stimulating hormone, etc.), leukocytes, sugars, heavy metals or toxins, bacterial components (e.g. proteins, sugars, or antigens specific to a particular type of bacteria, such as *E. coli*0157:H7, *Staph. aureus, Salmonella* sp., *Salmonella typhii, Shigella, C. perfringens, Clostridium difficile, Campylobacter, Helicobacter pylori, L. monocytogenes, V. parahaemolyticus, Vibrio cholerae*, or *B. cereus*), ova and parasites, and physical characteristics of the urine sample, such as pH and specific gravity. Any analyte can be detected for which a reliable assay can be designed. With reference to the present disclosure the person of ordinary skill in the art will realize a variety of antigens that can be detected using a variety of assay principles applicable in the invention.

Test Kits

[0057] A further aspect of the present invention provides kits containing one or more collection slides, and/or one or more assay devices of the present invention, and instructions for their use in carrying out an assay. The test kits can be packaged in a variety of formats, depending upon the needs of the user. In one embodiment the instructions provided with the kit are instructions for detecting the presence of hemoglobin in a stool sample.

[0058] In one embodiment, the kit contains three collection slides, three assay devices, three applicators, a desiccation mailing pouch having three sealable compartments, and instructions for collecting a sample, provided in a package. The package can be any suitable container. In various embodiments the package can be a box, a pouch, a bag, or can be simply a wrapping binding the items of the kit together.

[0059] In another embodiment the kits contain one or more collection slides and assay devices individually packaged in foil pouches, and one or more bottles of extraction buffer, and instructions, provided in a package. In another embodiment the kits contain three individually wrapped collection slides, extraction buffer for performing three tests, and instructions for use. At a health care facility where many tests would be conducted, the kit can contain many individually wrapped test devices, one or two large bottles of extraction buffer, and a single copy of the instructions.

[0060] A further embodiment provides a kit containing two "mini-kits," wherein one mini-kit contains packaged together three collection slides, three applicators, a desiccant mail pouch and instructions for the patient explaining how to correctly collect the samples. The second mini-kit would contain, packaged together for the doctor, three test devices, extraction buffer sufficient to perform three tests and instructions for use.

EXAMPLE 1

Use of the Collection Slide and Assay Device for Analysis of hHb in Stool

[0061] Six collection slides of the invention were loaded with stool sample by smearing sample onto the sample collection pad. After drying, each slide was placed into the docking area of an assay device of the invention, as depicted in the Figures. By placing the collection slides into the docking area, the hinged side of the slide was inserted under the tang, and the slide pressed downward and snapped into place in the docking area, so that the eluent orifice of the collection slide was in fluid communication with the absorbent transfer material of the device.

[0062] Three drops (about 200 μl) of extraction buffer were then applied to the buffer orifice of the collection slide. In all cases, within 7-16 seconds, the buffer had flowed through the sample collection pad and out of the eluent orifice, and into the sample well. Within about 50 seconds, the buffer had flowed onto and through each of the test strips, and lines appeared at the control lines. The test strip had a test line with specific binding molecules for hHb, and a reagent zone with labeled antibodies for hHb.

[0063] The invention illustratively described herein may be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by various embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0064] The contents of the articles, patents, and patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other documents.

1. A device for detecting an analyte in a sample, comprising:

a housing containing

a test element,

a docking area for receiving and engaging an external collection slide, the docking area comprising a sample receiving orifice having one or more fluid transfer structures comprised within the circumference of the sample receiving orifice; and

a results window for observing a test result.

2. The device of claim 1 wherein the sample receiving orifice is comprised in a well in the housing of the device.

3. The device of claim 1 wherein the one or more fluid transfer structures comprise a crossbar that projects above the plane of the docking area.

4. The device of claim 3 wherein the crossbar is positioned to be in fluid communication with an engaged collection slide.

5. The device of claim 1 wherein the docking area comprises one or more projections for securing the external sample collection slide in position above the sample receiving orifice.

6. The device of claim 5 wherein the one or more projections comprise one or more snap locks.

7. The device of claim 1 wherein the docking area is comprised as a depression in the housing and is at least partially circumscribed by a raised area of the housing.

8. The device of claim 1 wherein

the test element comprises a bibulous matrix having a sample application zone in fluid communication with the one or more fluid transfer structures;

a reagent zone comprising reagents for conducting an assay; and

a detection zone comprising a test line for visually detecting the presence or absence of the analyte at the test line.

9. The device of claim 8 wherein the test line further comprises a specific binding molecule for the analyte immobilized on the matrix.

10. The device of claim 9 wherein the specific binding molecule is an antibody.

11. The device of claim 9 wherein the specific binding molecule on the test line binds to human hemoglobin.

12. The device of claim 8 wherein the reagent zone comprises labeled specific binding molecule for the analyte.

13. The device of claim 1 wherein the analyte is human hemoglobin.

14. A method of detecting the presence or absence of an analyte in a sample contained in a sample collection slide, comprising:

placing a collection slide containing the sample into a docking area of a device for detecting analyte in a sample, wherein the device comprises:

a test element comprised within a housing;

a docking area for receiving a collection slide, the docking area comprising a sample receiving orifice having one or more fluid transfer structures comprised within the circumference of the sample receiving orifice; and

a results window for observing a test result; and

wherein the collection slide comprises

a first water resistant card having an eluent orifice;

a second water resistant card hingeably connected to the first card and having a solvent orifice, the collection slide having an open position and a closed position,

a sample collection surface present between the solvent and eluent orifices when the collection slide is in the closed position; and

applying an extraction buffer to the solvent orifice of the collection slide;

allowing the extraction buffer to pass through the sample area and through the sample receiving orifice and test element; and

observing a test result in the results window.

15. The method of claim 14 wherein the test element comprises,

a bibulous matrix comprising

a sample application zone in fluid communication with the one or more fluid transfer structures;

a reagent zone comprising reagents for conducting an assay; and

a detection zone comprising a test line for detecting the presence or absence of the analyte.

16. The method of claim 15 wherein the test line comprises specific binding molecules for the analyte.

17. The method of claim 15 wherein the test line contains reagents for conducting a chemical test.

18. The method of claim 14 wherein the analyte is human hemoglobin.

19. A kit for collecting a biological sample, comprising:

a device for detecting an analyte in a fluid comprising:

a housing containing

a test element,

a docking area for engaging a collection slide and comprising a sample receiving orifice having one or more fluid transfer structures comprised within the circumference of the sample receiving orifice;

a results window for observing a test result;

a sample collector;

an envelope for containing a loaded collection device; and

instructions for use; and

a collection slide comprising:

a first water resistant card having an inner surface and a eluent orifice;

a second water resistant card hingeably connected to the first card and having an inner surface and a solvent orifice, the collection slide having an open position and a closed position, wherein the solvent and eluent orifices are aligned when the collection slide is in the closed position; and

a sample collection area on the first water resistant card to which sample is applied for collection, present between the solvent and eluent orifices when the collection slide is in the closed position; and

a sample collector

provided in a package.

20. A kit according to claim 19 further comprising one or more bottles containing buffers for conducing an assay according to the instructions for use.

* * * * *

Exhibit C

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(54) **DEVICES AND METHODS FOR SAMPLE COLLECTION AND ANALYSIS**

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(57) **ABSTRACT**

The present invention provides devices, methods, and kits for the collection of a solid or semi-solid sample and analysis for the presence, absence, or quantity of an analyte. The invention provides a collection slide having a first card and a second card. The first card has a sample collection area. The first and second cards have orifices allowing the passage of fluid through the sample collection area, and the cards are hingeably connected to each other. The invention also provides an assay device having a housing with a test element, a results window, and a docking area for receiving and engaging the collection slide. In one embodiment the collection slide and device can be used to detect the presence of fecal occult blood (human hemoglobin) in a stool sample. Many other embodiments are described herein.

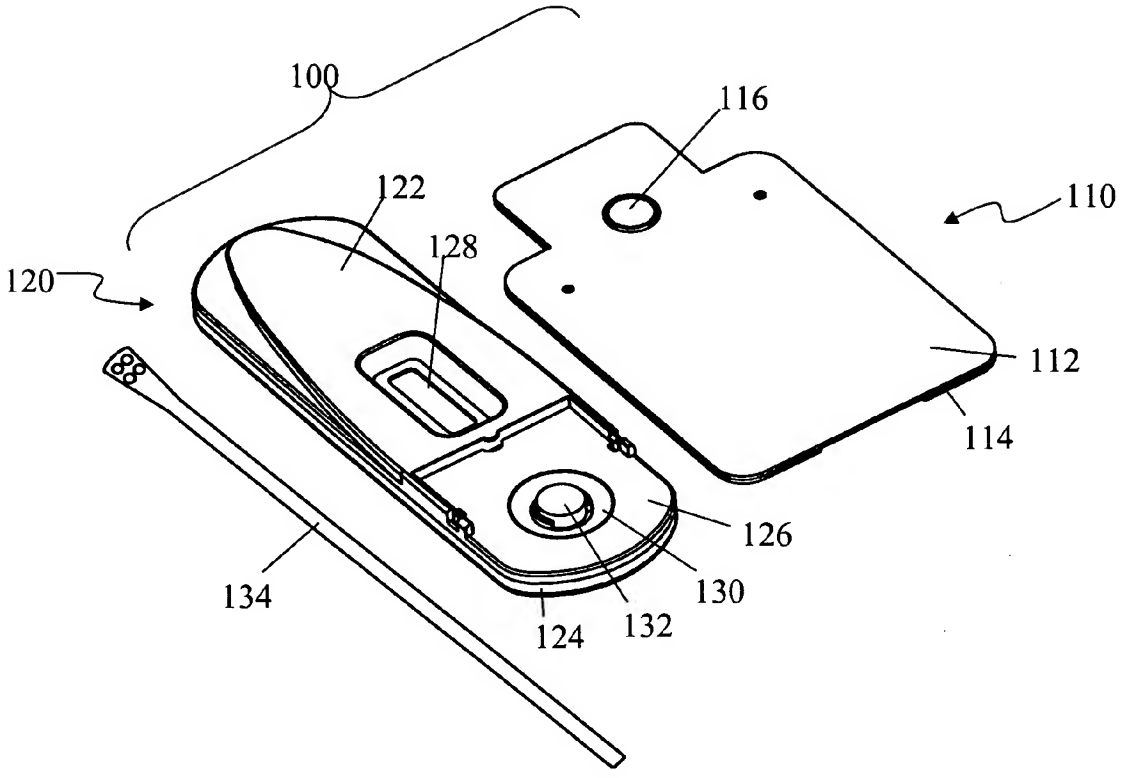

Figure 1

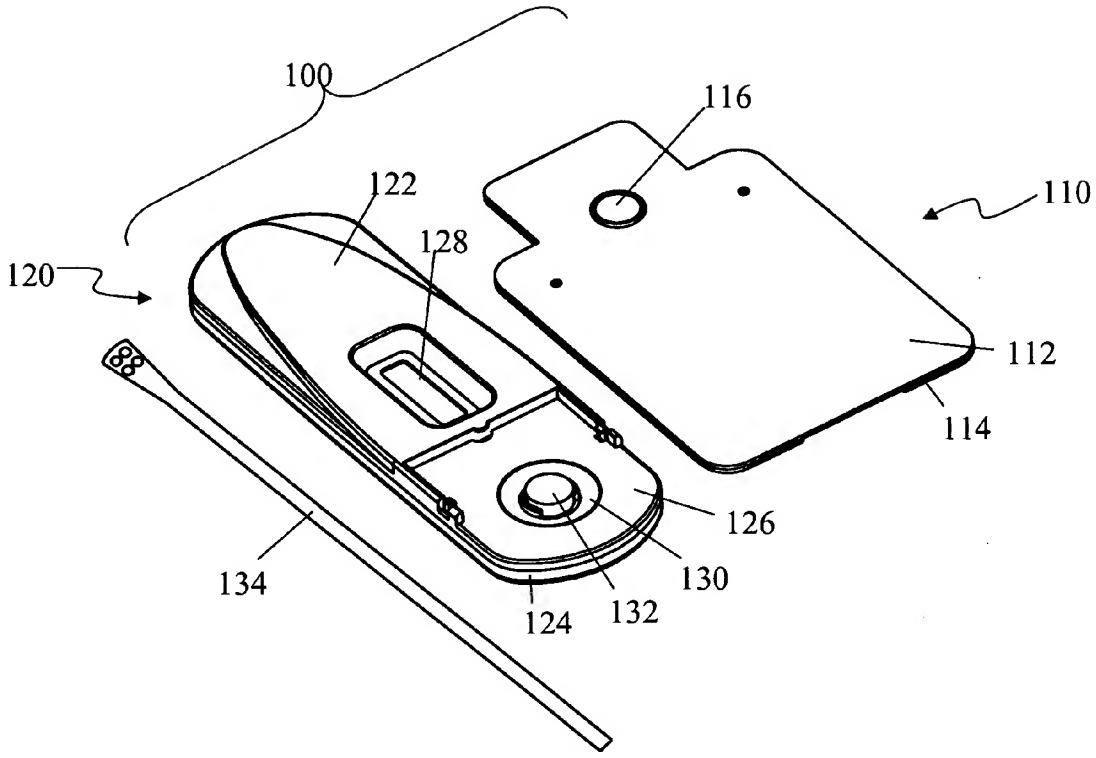

Figure 2

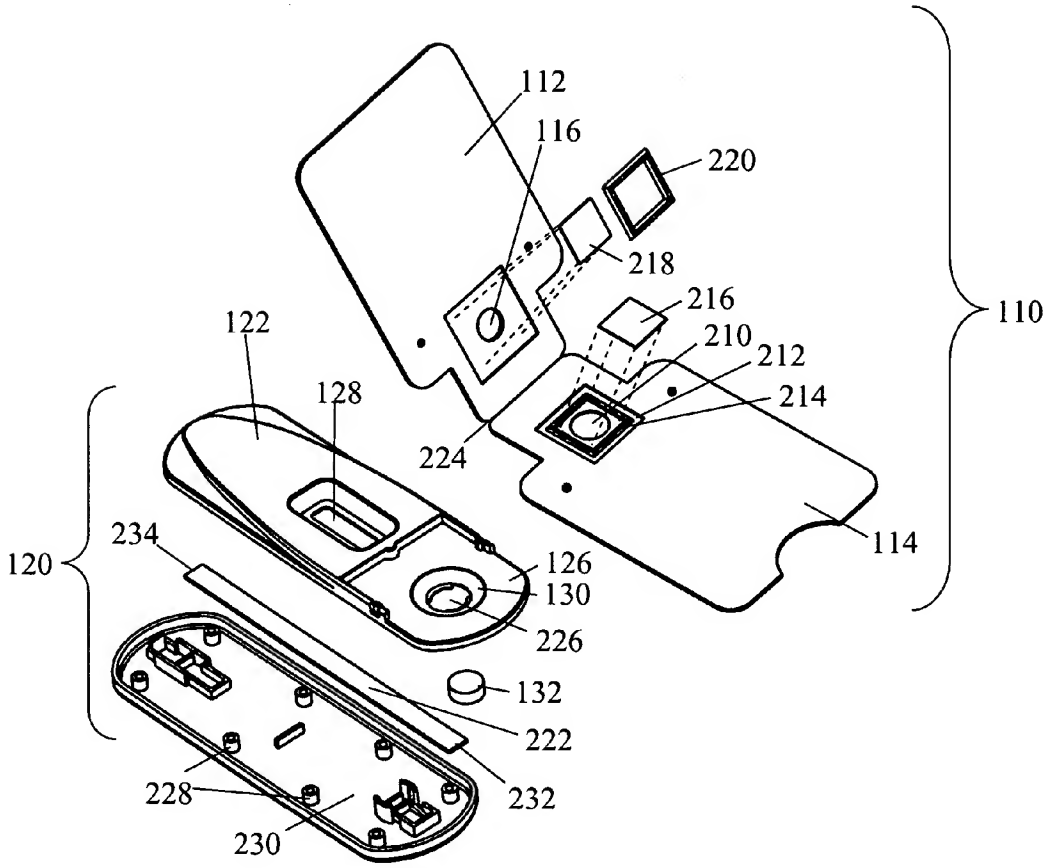
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Figure 3A

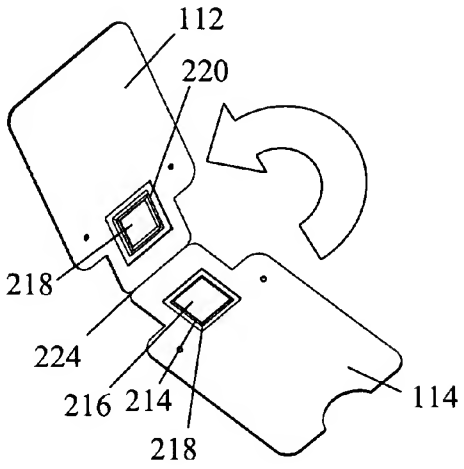


Figure 3B

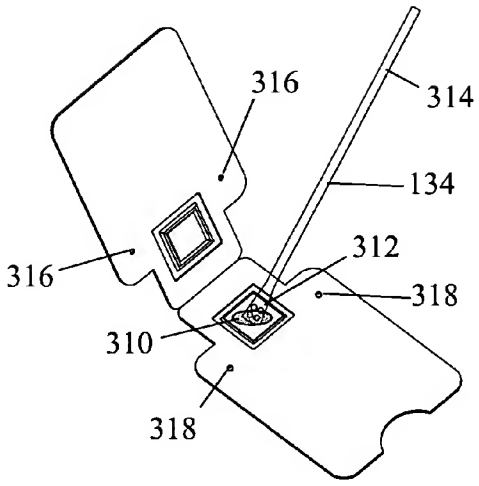


Figure 3C

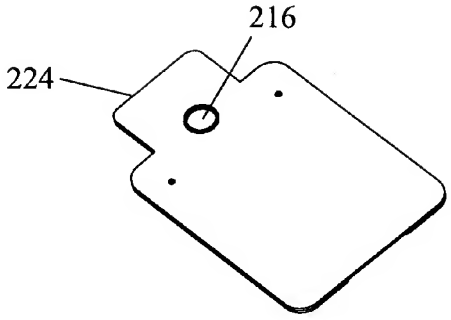


Figure 4

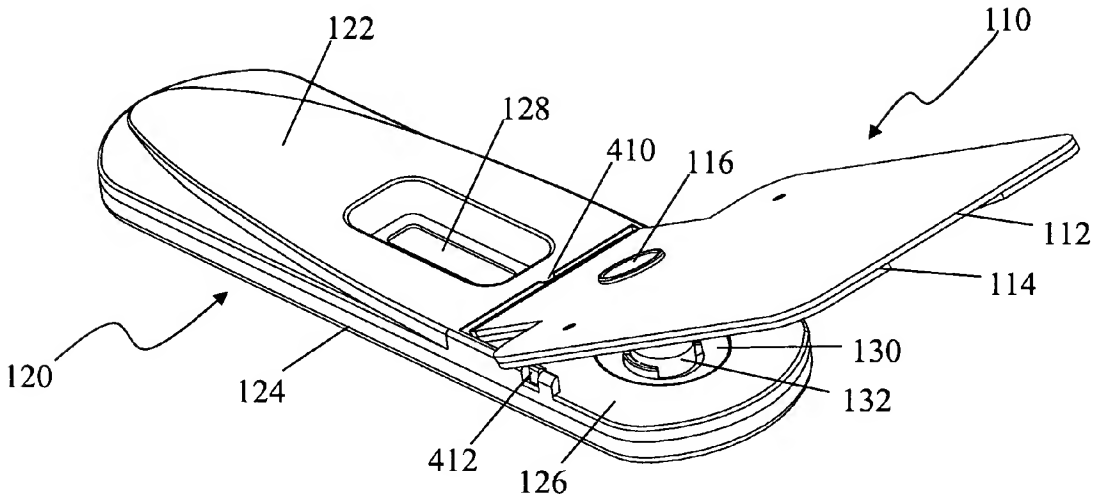


Figure 5

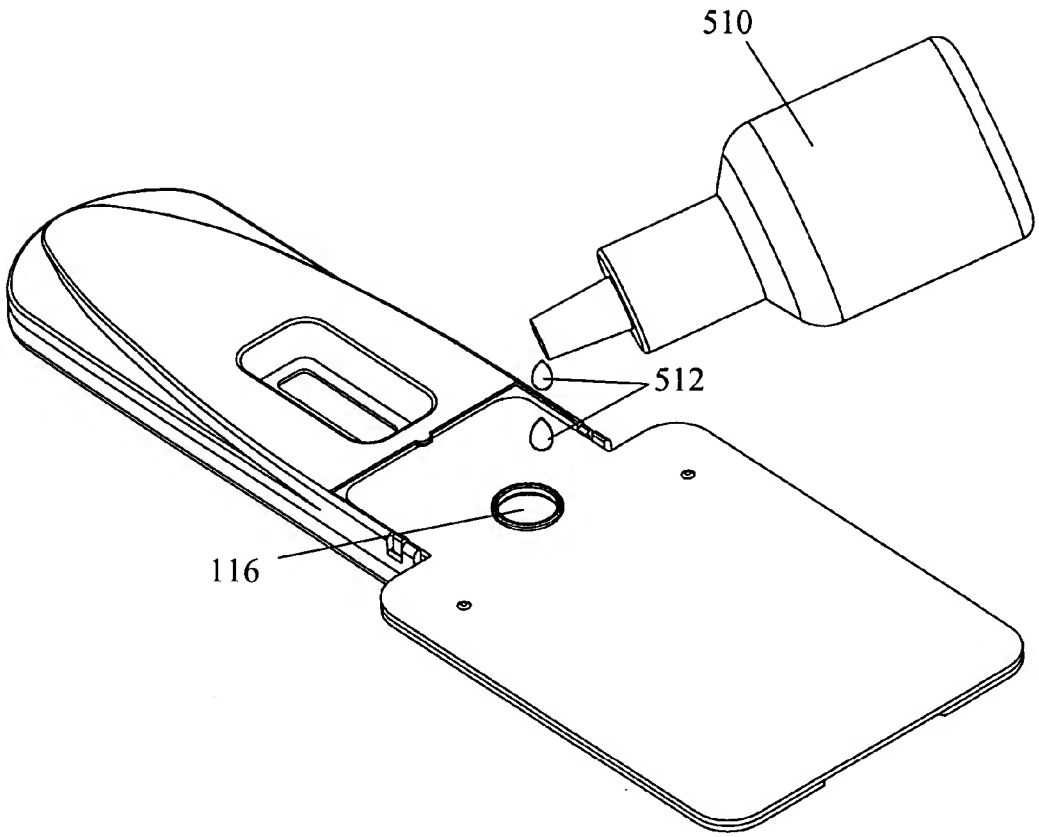
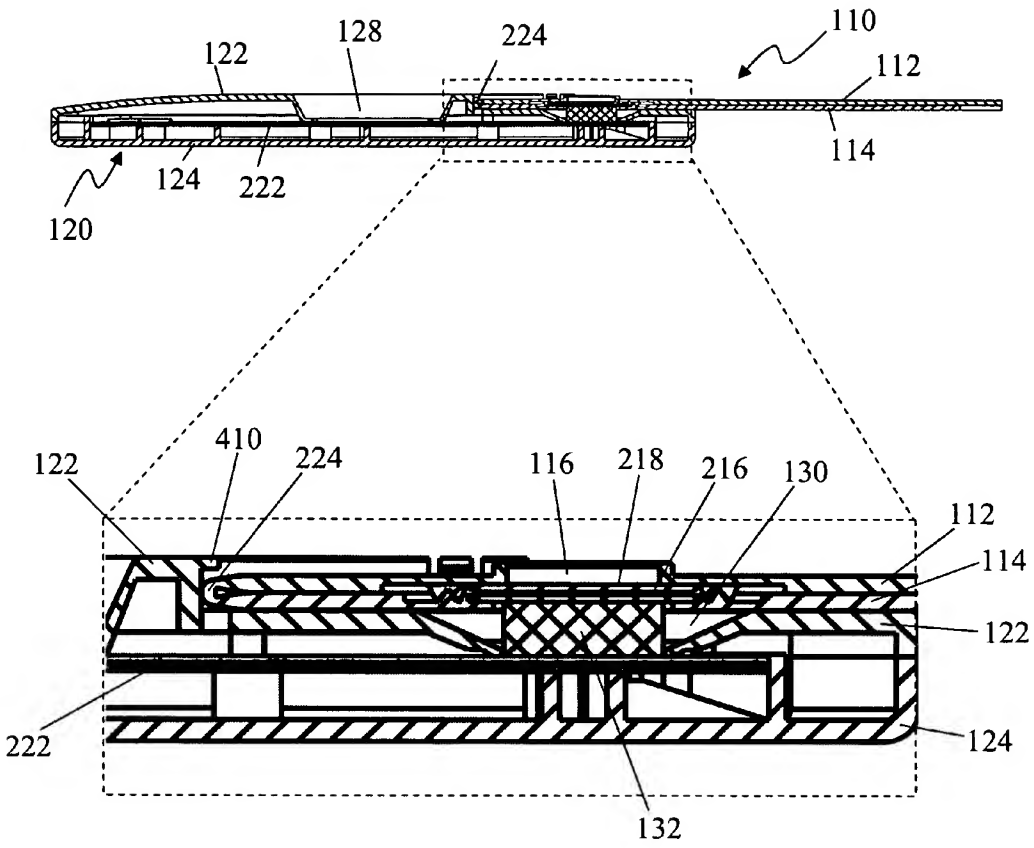


Figure 6


110
122
128
224
112
114
124
222
120
122
410
224
116
218
216
130
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114
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222
124
132

DEVICES AND METHODS FOR SAMPLE COLLECTION AND ANALYSIS

FIELD OF THE INVENTION

[0001] The present invention is directed to devices for the collection of solid or semi-solid biological samples, and their analysis for the presence of analytes.

BACKGROUND OF THE INVENTION

[0002] The following Background of the Invention is intended to aid the reader in understanding the invention and is not admitted to be prior art.

[0003] The detection of occult blood in stool samples is a preliminary method of detecting colon cancer. Traditional methods that detect hemoglobin in a stool sample, such as Guaiac-based chemical methods, are hampered by their inability to distinguish between dietary-derived hemoglobin (i.e. from meat in the diet) and human hemoglobin, which leads to a large number of false-positive test results. To over-come this difficulty, immunoassays specific for human hemoglobin (hHb) have been developed. The antibodies used in these assays are able to distinguish between hemoglobin derived from a human and that from another animal.

[0004] The collection and analysis of occult blood samples presents the problem of the unpleasantness of sample collection and analysis. Presently available devices fail to adequately solve these problems. Therefore, there is a clear and persistent need for a device that reduces the interaction of both the patient and the test operator with the sample while at the same time accurately detecting the presence of hHb in the sample.

SUMMARY OF THE INVENTION

[0005] The present invention provides devices, methods, and kits for collection and analysis of a biological sample. In one embodiment, the biological sample is a stool sample. One aspect of the invention is a collection slide having two cards that are hingeably connected. On the inner surface of a card is a sample collection area for deposition of the biological sample. The cards also contain orifices so that buffers can be passed through the cards and through the sample collection area, to elute analytes of interest from the sample contained in the cards. The present invention also provides a device for detecting an analyte in a biological sample. The device contains a test element, such as a test strip, having reagents for detecting the analyte. The device also contains a docking area for receiving the collection slide. In the docking area is a sample transfer orifice having an absorbent transfer material, which receives buffer passed through the orifices of the collection slide, and passes the eluted fluid to the test element.

[0006] In a first aspect the present invention provides a device for detecting an analyte in a sample. The device has a housing containing a test element, and a docking area on the housing for receiving and engaging a collection slide. The docking has a sample transfer orifice with an absorbent transfer bead disposed therein and in fluid communication with the test element. The device also has a results window for observing a test result.

[0007] The transfer material can be made of a variety of materials or shapes. In various embodiments the transfer material is ultra-high molecular weight polyethylene, polyethylene, polyurethane, nylon, polyester, polypropylene, polytetrafluoroethylene, or a cellulose-based material. In one embodiment, the transfer material is an ultra-high molecular weight polyethylene filter.

[0008] In one embodiment the sample transfer orifice of the device is a well situated in the housing of the device. The well is an indentation in the housing and serves to collect fluid that overflows from the absorbent transfer material. The absorbent transfer material can also contain reagents for improving the transfer of analyte from the collection slide to the test element. In one embodiment, the transfer material includes a reagent selected from the group consisting of: a surfactant, a protein, a buffer, a polymer and a preservative. A "surfactant" is a chemical compound that reduces the surface tension between two liquids. Surfactants can have a hydrophilic (attracted to water) group and a hydrophobic (repelled by water) group. "Proteins" are large molecules composed of one or more chains of amino acids in a specific order and folded shape determined by the sequence of nucleotides in the gene encoding the protein. A "buffer" is a solution containing either a weak acid and its salt or a weak base and its salt, and is resistant to changes in pH. A "polymer" can be any of numerous natural and synthetic compounds of usually high molecular weight consisting of up to millions of repeated linked units, each a relatively light and simple molecule. A "preservative" is an additive used to protect against decay, discoloration, or spoilage.

[0009] In another embodiment, the transfer material contains a reagent. In various embodiments the reagent can be any one or more of: a blocking agent, a surfactant, a wetting agent, a solubilizer, a stabilizer, a diluent and a preservative. "Blocking agents" can be any of a number of compounds or substances that bind to an analyte or a support media, and thereby prevent binding of an analyte to the support media. "Wetting agents" are usually organic based materials that modify the surface tension of liquids and help to provide a uniform coating on hard-to-wet or hydrophobic surfaces. "Stabilizers" are compounds that prevent degradation of the tertiary structure of compounds. For example, compounds can be added to the collection slide to prevent decomposition of the Hb molecules in the sample, or of specific binding molecules in the device.

[0010] In a further embodiment, the transfer bead comprises a reagent selected from the group consisting of BRIJ® 35, Chemal LA-9, Pluronic® L64, Surfactant 10G, Span® 60, Silwet® L7600, Rhodasurf ON-870, Cremohor® EL, Tween® 20, Tween® 80, Surhynol® 485, Igepal® CA210, Triton® X-45, Triton® X-100, Triton® X-305, Bio-Terge® AS-40, Standapol ES-1, Benzalkonium Chloride, Tetronic® 1307, Surynol® 465, Ninate® 411, Pluronic® F69, Zonyl® FSN 100, AEROSOL® OT 100%, Geropon® T77, sodium dodecylsulfate, sodium taurocholate, sodium cholate, CTAB, LDAO, CHAPS, NP40, n-octyl sucrose, n-dodecyl sucrose, n-dodecyl maltoside, octyl glucoside, octyl thioglucoside, n-hexyl glucoside, n-dodecyl glucoside, Tris(hydroxymethyl) aminomethane buffer, phosphate buffer, borate buffer, tartrate buffer, phthalate buffer, PVP K-30, PVP K-90, GANTREZ® AN-119, polyethylene oxide, polyelthylene glycol, PEG 800, GANTREZ® AN-119, polyvinylalcohol, PVP/VA S630, bony fish gelatin, crosslinked polyacrylic acid polymer, hydroxyporpylcellulose, sodium carboxymethylcelluose, sodium polystryenesulfonate,

sodium carageenin, acrylic latex, hydroxyethylcellulose, bovine serum albumin, egg white albumin, casein, ProClin® 300 and sodium azide.

[0011] In another embodiment, the test element is present within the housing and is a bibulous matrix having a sample application zone in fluid communication with the absorbent transfer material. The test element has a reagent zone containing reagents for conducting an assay, and a detection zone having a test line for visually detecting the presence or absence of the analyte at the test line. The test line can have a specific binding molecule for the analyte immobilized on the matrix. In one embodiment, the analyte of interest is human hemoglobin and the specific binding molecule on the test line binds to human hemoglobin. The reagent zone can contain a labeled specific binding molecule for the analyte, which in one embodiment is an antibody. The specific binding molecule can be present in a dried form, and can be solubilized by the passing sample fluid. In another embodiment the test line has reagents for conducting a chemical test.

[0012] In certain embodiments, the docking area has one or more snap locks for holding a sample collection slide in position in the docking area. For example, the docking area can have projections for securing a sample collection slide in position above the absorbent transfer pad. By "snap lock" is meant one or more projections through which the collection slide fits tightly. Thus, when the collection slide is moved into place, it will "snap" into position past the "snap locks." The projections can project into the area of the docking area. The docking area can also be an area into which the collection slide is inserted in a sliding motion. In either embodiment, the eluent orifice of the collection slide is brought into liquid communication with the absorbent transfer material. The docking area can be present as a depression or depressed portion of the housing, which is at least partially circumscribed by a raised area of the housing. Alternatively, the docking area can be present as a raised portion of the housing.

[0013] In another aspect, the present invention provides a collection slide for collecting and transferring a sample. The collection slide has a first card having an inner surface and a eluent orifice, and a second card hingeably connected to the first card and having an inner surface and a solvent orifice. The collection slide has an open position and a closed position, where the solvent and eluent orifices are aligned when the collection slide is in the closed position. The collection slide also has a sample collection pad on the first card, to which sample is applied for collection. In one embodiment the sample collection pad is present between the solvent and eluent orifices when the collection slide is in the closed position. The first and second cards can be made of a water-resistant or water-impermeable material. In one embodiment, the first and second cards are made of plastic.

[0014] In one embodiment, the sample collection area further has a collection pad overlaying the eluent orifice on the first card, with the sample application area at least partially circumscribed by a sealing structure on the first card. The second card can have a cover pad overlaying the solvent orifice, where the second card also has a sealing structure, complementary to the structure on the first card. In one embodiment, the structure on the first card is a gasket, which engages the structure on the second card, which is a groove, when the collection slide is in the closed position. By the two structures "engaging" is meant that a barrier is formed by their interaction which impedes the movement of sample into or out of the sample collection area. "Sealing structures" are those which impede the movement of sample into or out of the sample collection area when engaged. In an alternative embodiment, the first card can have the groove and the second card can have the gasket. Also, some embodiments utilize other structures, for example ridges that are generally sealed when the first and second card are in the closed position, or other structures. In a further embodiment, the first card or second card contains one or more holes for receiving a projection from the second card or first card, respectively, to retain the slide in a closed position. The cover pad and sample collection pad can be made of any suitable material. In some embodiments the cover pad and sample collection pad are made of a fibrous or bibulous material.

[0015] In another embodiment of the collection slide, the cover pad and/or collection pad contain reagents for eluting analyte from the sample. In certain embodiments, the reagent can be one or more of surfactants, buffers, proteins, polymers and preservatives, or a blocking agent, a surfactant, a wetting agent, a solubilizer, a stabilizer, a diluent and a preservative. Examples of useful reagents include BRIJ® 35, Chemal LA-9, Pluronic® L64, Surfactant 10G, Span® 60, Silwet® L7600, Rhodasurf ON-870, Cremohor® EL, Tween® 20, Tween® 80, Surhynol® 485, Igepal® CA210, Triton® X-45, Triton® X-100, Triton® X-305, Bio-Terge® AS-40, Standapol ES-1, Benzalkonium Chloride, Tetronic® 1307, Surynol® 465, Ninate® 411, Pluronic®° F69, Zonyl® FSN 100, AEROSOL® OT 100%, Geropon® T77, sodium dodecylsulfate, sodium taurocholate, sodium cholate, CTAB, LDAO, CHAPS, NP40, n-octyl sucrose, n-dodecyl sucrose, n-dodecyl maltoside, octyl glucoside, octyl thioglucoside, n-hexyl glucoside, n-dodecyl glucoside, Tris(hydroxymethyl) aminomethane buffer, phosphate buffer, borate buffer, tartrate buffer, phthalate buffer, PVP K-30, PVP K-90, GANTREZ® AN-119, polyethylene oxide, polyelthylene glycol, PEG 800, GANTREZ® AN-119, polyvinylalcohol, PVP/VA S630, bony fish gelatin, crosslinked polyacrylic acid polymer, hydroxypropylcellulose, sodium carboxymethylcellulose, sodium polystryenesulfonate, sodium carageenin, acrylic latex, hydroxyethylcellulose, bovine serum albumin, egg white albumin, casein, ProClin® 300 and sodium azide.

[0016] In another aspect, the present invention provides methods of detecting the presence or absence of an analyte in a sample contained in a sample collection slide. The methods involve placing a collection slide containing the sample into a docking area of a device for detecting analyte in a sample. The device and collection slide used in the methods are any as described herein. Additional steps of the methods involve applying an extraction buffer to the solvent orifice of the collection slide, allowing the extraction buffer to pass through the sample area and into the absorbent transfer bead and test element, and observing a test result in the results window.

[0017] In another aspect, the present invention provides kits for collecting and analyzing a biological sample. In one embodiment, the kits contain at least one collection slide as described herein, and a device for detecting an analyte in a fluid as described herein, provided in a package. In addi-

tional embodiments, the kits can contain one or more sample collector(s) as described herein, an envelope for containing a loaded collection device, and instructions for use, provided in a package. In various embodiments the kits can contain the sample collection slide, the device, and one or more of any of the additional components described. Any of the kits can also contain one or more bottles containing buffers for conducing an assay according to the instructions for use. In one embodiment the instructions for use are instructions for detecting the presence of hemoglobin in a feces sample.

[0018] In another aspect, the present invention provides methods of collecting a sample. The methods involve contacting a sample applicator loaded with sample with the sample collection pad of a collection slide as described herein, and placing the collection slide in the closed position. In some embodiments the methods also involve placing the closed collection slide containing the collected sample into an envelope or desiccation chamber. The placing of the collection slide into the closed position can include the step of pressing the first card and second card together to engage the one or more projections into one or more holes and locking the collection slide in the closed position. The placing the collection slide into the closed position can cause excess sample to be excluded from the sample collection pad.

[0019] In one embodiment the sample collection applicator is a tool having a portion for collecting sample, and the portion for collecting sample has a plurality of holes for the drainage of a fluid portion of the sample. In one embodiment, the portion for collecting sample is primarily flat.

[0020] The present invention includes a variety of other useful aspects, which are detailed herein. These aspects of the invention can be achieved by using the articles of manufacture and compositions of matter described herein. With reference to the present disclosure, it will be further recognized that various aspects of the present invention can be combined to make desirable embodiments of the invention. In addition, a variety of other aspects and embodiments of the present invention are described herein.

[0021] The summary of the invention described above is not limiting and other features and advantages of the invention will be apparent from the following detailed description, as well as from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] **FIG. 1** provides a perspective view of the different aspects of the present invention **100**, which includes a sample collection slide **110** and a test device **120** that engages the collection slide. Also shown is the sample collector **134** for applying the sample to the collection slide.

[0023] **FIG. 2** provides and exploded view of the devices shown in **FIG. 1**.

[0024] **FIGS. 3A-3C** illustrate application of a sample to the collection slide. **FIG. 3A** illustrates an opened collection slide, showing a cover pad **218** and a collection pad **216**. **FIG. 3B** illustrates application of the sample **310** to the collection pad. **FIG. 3C** illustrates a closed collection slide.

[0025] **FIG. 4** illustrates a collection slide **110** engaging the docking area **126** of a test device.

[0026] **FIG. 5** illustrates application of extraction buffer **512** to the solvent orifice **116** of the engaged collection slide.

[0027] **FIG. 6** provides a cross-sectional view of the collection slide **110** engaged in a test device **120**.

DETAILED DESCRIPTION

[0028] In the following detailed description, reference is made to the accompanying drawings that form a part hereof, and in which is shown by way of illustration specific embodiments in which the invention may be practiced. It is understood that other embodiments may be utilized and structural changes may be made without departing from the scope of the present invention.

Collection Slide

[0029] The present invention provides collection slides for collecting a solid or semi-solid sample. In some embodiments the sample is a biological sample, such as a stool sample. The present invention also provides devices for detecting the presence of analytes in the sample, and methods for collecting the sample.

[0030] With reference to **FIGS. 1-5**, the collection slide **110** has a first card **114** and a second card **112**. The first and second cards may be made of any appropriate material. For example, the cards can be made of a resilient, water resistant or water-impermeable material, such as plastic, coated cardboard, metal or glass. In one embodiment, the cards are hingeably connected to each other, for example by a hinge **224** (**FIG. 2**). By "hingeably connected" is meant that the two cards are connected to each other at their first ends and have free ends movable towards and away from each other by movement about the hinge. A wide variety of hinge connections may be advantageously used. In the exemplary embodiment shown in the figures, the collection slide is manufactured of injection molded plastic and the two cards are connected by a living hinge, as depicted in **FIG. 2**. In other embodiments, the hinge can be one or more flaps of material that bind the two cards together and allow for one card to be folded onto the other card. In another embodiment the cards are present as separate cards that can be secured together, for example by a locking mechanism. The second card has a buffer or solvent orifice **116**, through which an extraction buffer **510**, **512** can be applied to a collected sample (**FIGS. 1 and 5**).

[0031] The collection slide has an open position and a closed position (compare **FIGS. 1 and 2**). As illustrated in **FIG. 2**, the first card has an eluent orifice **210** and the second card has a solvent orifice **116**. The buffer and eluent orifices are positioned on the cards so that when the collection slide is in the closed position, the two orifices are in alignment. By the orifices being "aligned" or "in alignment" is meant that a liquid applied to the solvent orifice in the second (or top) card in sufficient quantity will pass through the sample collection area and through the eluent orifice.

[0032] Referring to **FIG. 2**, a cover pad **218** is present on the inner surface of the second card and overlaying the buffer orifice **116**. The cover pad and sample collection pad can be made of any suitable material that retains sample and allows the passage of fluid. Examples of materials suitable for the cover pad and/or sample collection pad are polyester mesh, fibrous or bibulous materials, paper or paper-based materials, synthetic fabrics, meshes and wools, coated or

supported papers, polyesters, nylon membranes, nitrocellulose, glass wool, treated paper, absorbent paper, or a material made of a cellulose base. In the embodiment shown, the cover pad **218** is circumscribed by a gasket **220**. With reference to the present disclosure the person of ordinary skill in the art will realize many other materials suitable for the cover pad and/or sample application pad.

[0033] On the first card is present an eluent orifice **210**, which is overlaid with a sample collection pad **216**. The sample collection pad **216** can be made of any suitable material that retains sample and allows for the passage of fluid. In various embodiments the sample collection pad **216** is made of the same types of materials as the cover pad. The sample collection pad can be circumscribed by ridge **214** and groove **212**, or by a series of ridges and grooves. The cover pad and the collection pad can be made of any suitable material that retains sample and allows for the passage of fluid. Examples are provided above with respect to material for the cover pad. The material should also have sufficient resiliency to withstand the mechanical pressure of the sample application. Preferably, the material does not deteriorate or tear when wet.

[0034] Common difficulties with stool sample collection include that patients tend to over-apply sample to collection slides, which can cause interference when the assay is an immunoassay. The collection slide of the present invention limits the amount of sample that can be applied to the slide while requiring no direct sample manipulation by the technician conducting the test. The amount of sample collected is limited to the sample collection area, since the cover pad and sample collection pad are circumscribed by the sealing structures (e.g., a gasket and groove) when the slide is in the closed position. When the collection slide is moved to the closed position, the interaction of the sealing structures (e.g., the interaction of the gasket with the groove and ridge) separates the sample within the sample application area from sample applied outside the sample area. After the sample has been applied to the sample collection area, the collection slide is closed and retained in a locked position, thereby limiting the volume of sample retained within the sample area, because excess sample is squeezed out as the two cards are pressed together. The sealing structures can also be structures other than a gasket, ridge, and groove. For example, the structures can be a pressure sensitive adhesive or a wax bead (or beads) present on or around the sample collection pad and/or cover pad, which seal the sample collection pad when the two cards are closed and pressed together. The "seal" does not have to be a tight seal, just that it generally impedes the passage of sample into or out of the sample collection area when the collection slide is in the closed position. With reference to this disclosure the person of ordinary skill will realize many other structures that will find use in other embodiments of the invention.

[0035] The cover pad and/or collection pad can be treated with reagents that improve the flow of aqueous liquids through them. Additionally, these treatments also improve the elution of the analyte of interest from the dried sample within the sample area. In one embodiment the pads are treated with surfactants to inhibit proteins from sticking to the pads and to promote protein solubilization. A wide variety of commonly used anionic and non-ionic surfactants may be advantageously used in various concentrations. Some cationic and amphoteric surfactants may also find use in the present invention. Some examples of surfactants that may be used to treat the pads include, but are not limited to, the polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (e.g., the BRIJ® (ICI US, Inc.) series of surfactants). Other useful surfactants include octyl phenol ethoxylate surfactants (e.g., polyethyrene glycol mono-p-iso-octylphenyl ether and other Triton® (Rohm & Haas, Philadelphia, Pa.) series surfactants), polyoxyethylene derivatives of sorbitan esters (e.g., the Tween® (ICI Americas, Inc.) series of surfactants) and block copolymers based on ethylene oxide and propylene oxide and represented by $HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$ (.e.g., the Pluronic® (BASF) series of surfactants). With reference to the present disclosure, a surfactant can be conveniently chosen using known surfactant selection techniques, such as by using a commercially available surfactant tool kit, for example, the Reagent Developer's Surfactant Took Kit (Pragmatics, Inc., Elkhart, Ind.), or a similar kit. These kits provide a convenient method of testing a large number of surfactants on a specific application, in order to optimize protein extraction and flow-through.

[0036] In some embodiments the pads may be treated with a buffer containing a component that improves analyte stability. Buffers can also condition the sample to promote optimal binding between the analyte and the specific binding reagents (e.g., antibodies or antibody fragments), which can be utilized in the assay. This can be performed, for example, by adjusting the pH of the analyte. Buffers having these useful qualities include, but are not limited to, Tris(hydroxymethyl) aminomethane buffer, phosphate buffer, borate buffer, tartrate buffer and phthalate buffer.

[0037] A "specific binding molecule" refers to a molecule that binds to a target analyte (e.g., human hemoglobin) and does not substantially bind to any other molecule present in the sample. In some embodiments a specific binding molecule can also bind to a molecule that correlates with or indicates the presence of an analyte of interest in a sample. By substantial binding is meant that binding occurs to an extent that will affect the result of an assay performed with the specific binding molecules, i.e., a less optimal or less accurate result will be obtained. A small amount of non-specific binding that may occur and that does not change the result of the assay is not considered substantial binding. In some embodiments the specific binding molecule can be an antibody or an antibody fragment (e.g., the Fab region of an antibody), an antigen, a receptor or fragment of a receptor that binds a ligand, or a member of a biotin-streptavidin pair or other type of binding pair.

[0038] The cover pad and/or sample application pad can also be treated with one or more polymers, which can also have the property of improving analyte stability and elution. Polymers sometimes used in protein purification can be useful for this purpose. Examples of useful polymers include, but are not limited to, polyvinylpyrrolidone (PVP), poly(methylvinylether-co-maleic anhydride, polyethylene oxide (PEO), polyelthylene glycol (PEG), copolymers of methyl vinyl ether and maleic anhydride (e.g., poly(methylvinylether-co-maleic anhydride), polyvinylalcohol (PVA), vinylpyrrolidone/vinylacetate, bony fish gelatin (from fish of the class Osteichthyes), crosslinked polyacrylic acid polymer, hydroxypropylcellulose (HPC), sodium carboxymethylcelluose (CMC), sodium polystyrenesulfonate, sodium carageenin, acrylic latex, and hydroxyethylcellulose

(HEC)). These polymers are commercially available (e.g., from Pragmatics, Inc., Elkhart, Ind.), and are conveniently formulated in a polymer tool kit. They can therefore be used systematically to determine the advantages of particular polymers in particular applications.

[0039] To improve analyte extraction, the pads may also be treated with a non-specific protein, which functions as a blocking agent. Any protein may be used for this purpose including, but are not limited to, bovine serum albumin, egg white albumin, and casein.

[0040] The cover pad and sample application pad can also be treated with a preservative to increase the shelf-life of the collection slide. A "preservative" is a naturally or synthetically produced chemical added to inhibit microbial growth or undesirable chemical changes. Any preservative can be used that provides the preserving effect and does not interfere with the assay. Examples of useful preservatives include, but are not limited to, 5-chloro-2-methyl-isothiazol-3-one (e.g. ProClin® 300 (Supelco, Inc., Bellefonte, Pa.) and sodium azide. With reference to the present disclosure the person of ordinary skill will realize many other preservatives that will find use in the present invention.

[0041] The cover pad and collection pad form the top and bottom walls of the sample collection area, and serve to eliminate excess sample from the sample collection area. When the structures on the cards are a gasket, ridge, or groove, they can also be situated on the opposite cards as those described above.

[0042] In certain embodiments, one of the cards of the collection slide is provided with structures for securing the first and second cards in a closed position. In one embodiment short pins **316** (**FIG. 3B**) are present on the interior surface of one card. The opposite card is provided with holes **318** that mate with the pins. When the collection slide is closed, the pins are inserted into the holes and lodged with sufficient resistance to hold the collection slide in a closed or "locked" position. In one embodiment this action may advantageously cause a snapping noise, alerting the patient that the collection slide has been properly closed. Other methods of securing the collection slide in a closed position can also be incorporated into the slide. For example, a clip that fits over the outside of the two cards and holds them together could be used in one embodiment, or snaps present on the inner surfaces of the two cards can be used in another embodiment. With reference to the present disclosure the person of ordinary skill will realize other structures for retaining the collection slide in the closed position.

Sample Collector

[0043] The present invention also provides a sample collector **134** (**FIG. 1**). The sample collector has a handle **314** (**FIG. 3**) and a spatula **312** for moving the sample. In one embodiment the spatula is perforated with a plurality of holes, which reduces the liquid content of the sample, and also serves to reduce application of excess sample to the sample collection pad. In various embodiments the spatula portion of the device is perforated with 4, 5, 6, 7, 8, 9, 10, 11, 12, or more holes. The spatula portion of the collector can be generally flat, or can have a curved (spoon-like) shape. This device can be made of any suitable material (e.g., plastic). In one embodiment, the spatula portion of the device is made of a soft plastic, and the handle is made of a harder plastic. This will enable the spatula to bend when sample is applied to the sample collection pad and lay on the pad. The perforations in the spatula portion will also act as an aid in applying an even sample to the pad.

Methods of Collection

[0044] Another aspect of the present invention is methods of collecting a sample. In one embodiment the sample is a stool sample. The method of sample collection and operation of the collection slide and assay device is illustrated in **FIGS. 3A-3C**.

[0045] One embodiment of the methods is illustrated in **FIG. 3A**. The patient opens the collection slide to expose the inner surfaces of the first and second slides, revealing the cover pad and sample collection pad. A small amount of stool sample is applied to the sample collection pad **216**. The collection slide is then closed (**FIG. 3C**). The present collection slide eliminates excess sample by providing a sample collection area, with a design such that only sample in the sample collection area will be incorporated into the assay. When the collection slide is closed, a structure the first card engages a structure second card, forming a wall that circumscribes the sample collection area. In one embodiment the structure on one card is a gasket, and the structure on the opposite card is a groove and a ridge. When the collection slide is in the closed position, the solvent or buffer orifice, the sample area, and the eluent orifice are all vertically aligned. In this position, when buffer is applied to the buffer orifice, it flows through the cover pad and into the sample collection area, and then out of the eluent orifice, thereby rinsing the sample in the process and solubilizing analyte of interest contained in the sample. Additionally, the buffer dilutes the sample and conditions it for optimal binding of analyte by the specific binding reagents on the test element. After passing through the eluent orifice, the liquefied sample is then passed into the absorbent transfer bead of the assay device.

[0046] It is known that human hemoglobin breaks down rapidly when left in a wet sample. To prevent analyte degradation, the methods can incorporate the step of drying the sample. This step can involve leaving the collection card exposed to air for a certain period of time to allow it to air dry, or drying the sample in an oven at 45° C. The step can also involve placing the closed collection slide into a container containing desiccant. The container can be a sealable pouch (e.g., a mailing pouch). After drying (or placing the collection slide in a sealable pouch containing a desiccant), the collection slide can be presented to a health care facility for analysis.

Assay Device

[0047] Another aspect of the present invention is an assay device **120** for analyzing a sample in the collection slide for the presence or absence of an analyte of interest (see **FIGS. 1 and 2**). One embodiment of the assay device is shown in **FIGS. 1 and 2**. In this embodiment the assay device has a housing consisting of a top portion **122** and a bottom portion **124**, which engage one another and lock together. The housing may be constructed of any suitable material such as, for example, plastics, pressed hardboard, metals, ceramics, polymers (e.g., polycarbonate, polypropylene, cycloolefins), and other materials. In the embodiment illustrated in the Figures, the housing is made of molded plastic. The top and

bottom portions can engage one another by any convenient means, such as parts that snap together, glue, micro-welding, and other means. In the embodiment illustrated in **FIG. 2**, the top portion has a series of pins on the inner surface (not shown) which snap-fit snuggly into a corresponding series of raised rings **228** on the inner surface **230** of the bottom portion, thereby securing the top and bottom portions of the assay device in a locked position.

[0048] A docking area **126** for receiving and engaging a collection slide is located on the assay device. The collection slide may be "loaded" meaning that it contains a sample to be analyzed. The docking area may be of any shape, and can mate with a portion of the collection slide carrying the sample collection area. In one embodiment the docking area can receive and engage an external collection slide. An external collection slide is one that can be loaded separated from the assay device, and is not physically connected to the device at the time of sample loading. By "receiving and engaging" a collection slide is meant that the assay device and collection slide are placed into the "test position." The "test position" is when the sample application pad and absorbent transfer material are in liquid communication.

[0049] The docking area can also receive the collection slide in reversible fashion, meaning that the collection slide can be removed from the device after buffers are applied and sample eluted from the collection slide. As illustrated in **FIG. 4**, in this embodiment the collection slide is snapped into the docking area by fitting the hinged edge of the collection slide under a tang **410**. The collection slide is then pressed down onto the docking area and snapped into a locked position under one or more projections **412**. The projections hold the collection slide flush with the docking area. In other embodiments the docking area is slided into the assay device. In one embodiment the docking area can have a part that fits over the collection slide to hold it in place. When in place, the sample collection pad and the absorbent transfer material are in fluid communication. The buffer orifice is exposed to receive buffer, and buffer applied to the buffer orifice passes through the sample collection pad and into the absorbent transfer material. In one embodiment the docking area is configures to receive the collection slide against an exterior surface of the assay device, so that the sample collection area and absorbent transfer member are brought into liquid communication. The docking area can have projections for holding the collection slide securing in the test position.

[0050] In other embodiments the docking area can receive the collection slide into the interior of the device. In another embodiment the sample transfer orifice is the only orifice in the assay device for receiving sample or assay fluids, and the sample and assay fluids both the device through the sample transfer orifice. "Assay fluids" refers to buffers or other regents utilized during the assay. Thus, in these embodiments the sample transfer orifice is the sole orifice for receiving sample and fluids into the device.

[0051] As illustrated in **FIG. 1**, in one embodiment the docking area contains an indentation or well **130** having a transfer material **132** disposed therein. The absorbent transfer material can take any form, for example, a bead, cube, cylinder, oval, or any shape, and can be situated inside the well. As shown in **FIG. 2**, the transfer material protrudes through the top portion of the housing, through an orifice **226** to lie generally flush with or slightly protruding through the plane of the docking area. In this embodiment the absorbent transfer material is an absorbent transfer bead. Referring to **FIG. 6**, when the collection slide is snapped into the docking area, the buffer orifice, cover pad, sample collection pad, eluent orifice, and absorbent transfer bead are all generally in vertical alignment with each other. In this embodiment the absorbent transfer bead projects into or through the plane of the docking area, so that the absorbent transfer bead and the outer surface of the sample collection pad are placed into fluid communication through the eluent orifice. By being in "fluid communication" is meant that fluid passing through the sample collection area and through the sample collection pad is passed into the absorbent transfer material. The sample collection pad and absorbent transfer material may make direct physical contact, or be slightly apart from one another, but are retained in fluid communication.

[0052] The absorbent transfer material can be constructed of a variety of useful absorbent materials. The material should allow the transport of liquid from the collection slide to the test element of the assay device without changing the sample in a manner that interferes with the assay result. Examples of materials suitable for the absorbent transfer material include, but are not limited to, filter paper or other paper-based filter materials, nylon mesh filters, cellulose filters (or filters made of a cellulose-based material), polyester filters, and glass wool filters. In other embodiments the absorbent transfer bead is made of ultra-high molecular weight polyethylene (UHMWPE), polyethylene, polyurethane, nylon, polyester, polypropylene, or polytetrafluoroethylene. In a further embodiment, the transfer material is a filter made of ultra-high molecular weight polyethylene filter.

[0053] In various embodiments the absorbent transfer material is treated with reagents that improve the transfer of analyte from the collection slide to a test element of the device. The transfer material can be treated with any of the reagents described herein with respect to treatment of the cover pad and sample collection pad of the collection slide. Examples of reagents that can be used to treat the cover pad, sample collection pad, and absorbent transfer material include, but are not limited to, polyoxyethylene (23) dodecyl ether, polyoxyethylene (9) lauryl alcohol, poly(oxyethylene-cooxypropylene) block copolymer, p-isononylphenoxy-poly(glycidol), sorbitol anhydride monostearate, polydimethylsiloxane methylethoxylate, polyethoxylated (20) oleyl alcohol, polyethoxylated (35) castor oil, polyoxyethelene (20) sorbitan monolaurate, polyoxyethelene (20) sorbitan monolaurate, octylphenol ethoxylate (1.2), octylphenoxy-polyethoxy (5) ethanol, octylphenoxypolyethoxy (9-10) ethanol, octylphenoxypolyethoxy (30) ethanol, sodium olefin (C_{14}-C_{16}) solfonate, sodium polyoxethylene(1)lauryl sulfate, benzalkonium chloride, ethylenediamine alkoxlate block copolymer, 2,4,7,9-tetramethyl-5-decyne-4,7-diol ethoxylate (10), 2,4,7,9-tetramethyl-5-good wetter decyne-4,7-diol ethoxylate (30), amine alkylbenzene sulfonate, poly(oxyethylene-co-oxypropylene) block copolymer, telomer B monoether, sodium dioctylsulfo-succinate, poly-(vinylmethylether/maleic anhydride) copolymer, sodium N-oleyl-N-methyltaurate, dodecylsulfate, sodium taurocholate, sodium cholate, N-cytltrimethylammonium bromide, N,N-dimethyldodecylamine N-oxide, 3-[3-(cholamidopropyl)dimethylammonio]-1-proanesulfonate, alcohol ethoxylate, n-octyl sucrose, n-dodecyl sucrose, n-dodecyl maltoside, octyl glucoside, octyl thioglucoside, n-hexyl glucoside, n-dodecyl glucoside, tris(hydroxymethyl) aminomethane buffer, phosphate buffer, borate buffer, tartrate buffer, phthalate buffer, polyvinylpyrrolidone homopolymer, poly(vinyl-methylether/maleic anhydride),, polyethylene oxide, poly-

ethylene glycol, polyvinylalcohol, 1-ethenyl-2-pyrrolidinone, bony fish gelatin, crosslinked polyacrylic acid polymer, hydroxypropylcellulose, sodium carboxymethylcelluose, sodium polystyrenesulfonate, sodium carageenin, acrylic latex, hydroxyethylcellulose, bovine serum albumin, egg white albumin, casein, 5-chloro-2-methyl-isothiazol-3-one and sodium azide.

[0054] As illustrated in **FIGS. 2 and 6**, a test element **222** is provided with the housing, and in this embodiment is contained within the housing. The test element can be permanently situated within the housing of the device, meaning that it is not removable or insertable in conducting the assay, but is an integral part of the assay device. Referring to **FIG. 6**, the absorbent transfer bead is in fluid communication with the test element. In one embodiment, the test element is a bibulous test strip suitable for performing a lateral flow assay. A variety of test strips are suitable for use in the assay device. In one embodiment the test strips consist of a bibulous matrix, for example nitrocellulose, and/or other suitable materials. The matrix can have a sample loading zone, a reagent or label zone, and a detection zone. These types of test strips are known in the art and, with reference to the present disclosure, the person of ordinary skill will realize the variety of test strips that are useful in the present invention. In some embodiments a sample loading zone is present at one end of the test strip for the application of sample to the test strip. The sample loading zone is the portion of the test strip in liquid communication with the transfer material. Reagents for conducting the assay or conditioning the sample can also be present at the sample loading zone, or they can be present in a separate reagent or label zone. These reagents can serve a variety of purposes, for example preparing the sample for optimal binding with a specific binding molecule, or improving the stability of an analyte of interest. By "conditioning" a sample is meant adjusting the characteristics of the sample to promote or improve the reaction that detects the presence of the analyte. For example, buffers may be included to adjust the pH of the sample. If the sample contains substances that compete for binding with a specific binding molecule used in the assay, a secondary blocking antibody can be included to bind the substance, or if enzymes that would degrade the specific binding molecules for the analyte are present in the sample, one or more enzyme inhibitors can be added to the reagent zone.

[0055] The sample loading zone is present at the upstream end **232** of the test strip. Towards the downstream end of the test strip **234** is the reagent zone, which is followed by a detection zone. The reagent zone can include reagents for conditioning the sample, reagents for labeling the analyte (e.g., specific binding molecules if the assay is a sandwich format immunoassay) or labeled analyte analogs (e.g., if the assay is a competitive format immunoassay). In some embodiments the reagent zone contains a labeled specific binding molecule for the analyte present on the matrix in a dried form, and which can be solubilized by sample fluid as it passes along the matrix. In one embodiment the specific binding molecule is an antibody or fragment thereof. In one embodiment the analyte is human hemoglobin (hHb), and the labeled specific binding molecule is an antibody that binds hHb. The antibody can be labeled by any suitable methods, for example, a metal sol, colored latex beads, and dyes. In some embodiments the sample loading zone and the reagent zone over-lap. In other embodiments there are present a series of reagent zones located on the test strip.

[0056] The detection zone is the area of the test strip where the presence of the analyte is detected. In some embodiments the detection zone contains a test line for visually detecting the presence or absence of the analyte of interest at the test line. The test line can be of any shape, and need not be only a line. The test line can have a specific binding molecule for the analyte. When human hemoglobin is the analyte of interest, the specific binding molecule on the test line binds to hHb. In this embodiment the specific binding molecule binds to human Hb, and does not bind to hemoglobin that might be present from the diet, in order to avoid false positive results.

Methods of Detection

[0057] Another aspect of the present invention provides methods of detecting the presence or absence of an analyte in a sample contained in a sample collection slide. In one embodiment of the present method, a collection slide containing the sample is placed into the docking area of an assay device, as shown in **FIG. 4**. Extraction buffer **512** is applied to the buffer or solvent orifice of the collection slide. The extraction buffer elutes the analyte of interest from the sample, if the analyte is present. Buffer applied to the buffer orifice flows through the cover pad and into the sample collection area containing the dried sample. The dried sample is rehydrated and a portion of the sample elutes out of the collection slide, through the eluent orifice. In one embodiment the buffer is pulled through the collection pad and into the absorbent transfer material by capillary action. Excess buffer eluted from the collection slide is collected in the well surrounding the absorbent transfer material. Eluate within the transfer material flows by capillary action into the application zone of the test strip, and then to the downstream end of the test strip. As the eluate flows from the transfer material into the test strip, excess eluate held in the well may be absorbed and transferred to the test strip, by the transfer material.

[0058] As the eluate flows through the sample loading zone and reagent zone of the test strip, it dissolves reagents for conducting the assay present in the loading zone or reagent zone. In one embodiment these reagents are dried on the test strip. Reagents can also be included that condition the eluate for optimal detection, as described above. For example, if the assay is a sandwich format immunoassay reagents may include specific labeled binding molecules for the analyte, such as an antibody or fragment thereof. In one embodiment the specific binding molecule is a gold-labeled anti-hHb antibody or antibody fragment. If the analyte is present in the sample, the labeled specific binding molecule would capture the analyte and form a labeled, soluble complex, which is detected in the detection zone. The eluate continues to flow through the test strip to the detection zone, which contains a test line having specific binding molecules for the analyte. For example, the specific binding molecule can be an unlabeled antibody against the analyte, which binds at an epitope different from that of the labeling reagent. If the assay is a sandwich assay, the specific binding molecule in the test line captures the labeled antibody-analyte complex, and forms a visually detectable line indicating that the analyte is present in the sample. The test result therefore appears in the results window **128** located in the top portion of the housing.

[0059] In another embodiment the assay is a competitive format immunoassay. In this embodiment, the label zone or reagent zone of the test strip contains a labeled analog of the analyte, such as a gold-labeled hHb analog. If no analyte is present in the sample, the labeled analyte analog binds the antibody on the test line. Therefore a positive result on the

test line indicates that no analyte is present in the sample. When analyte is present, it competes with the labeled analog to bind the antibody on the test line. As the concentration of analyte in the sample increases, the amount of analog that binds to the test line decreases. Therefore, a lighter line or no line indicates the presence of analyte in the sample.

[0060] A procedural control can also be included in the detection zone. The procedural control can be present as a line, and will always appear whether or not analyte is present in the sample. Absence of a positive result from the procedural control indicates an invalid assay.

[0061] In other embodiments the eluate is tested by means other than an immunoassay. For example, the analyte-containing eluate could be detecting using a chemical means, such as a Guaiac test or other chemical means.

Types of Samples and Analytes

[0062] A "sample" is any material to be tested for the presence, absence, or quantity of an analyte. In one embodiment the sample is a biological sample, such as a stool sample. But any type of sample can be assayed using the present invention, as long as it contains an analyte to be detected that can be solubilized and can be passed through the collection slide and into the assay device. The sample can be in many forms, such as solid, semi-solid or highly viscous materials, such as stool, soils, tissues, blood, bodily fluids, or macerated organs. The sample may also be an oral or vaginal swab.

[0063] A variety of analytes may be tested for using the present device. Examples of analytes that can be detected using the present invention include, but are not limited to, hemoglobin or other blood components, creatinine, bilirubin, nitrite, protein (nonspecific), hormones (e.g. human chorionic gonadotropin, luteinizing hormone, follicle stimulating hormone, etc.), leukocytes, sugars, heavy metals or toxins, bacterial components (e.g. proteins, sugars, or antigens specific to a particular type of bacteria, such as *E. coli*0157:H7, *Staph. aureus, Salmonella* sp., *Salmonella typhii, Shigella, C. perfringens, Clostridium difficile, Campylobacter, Helicobacter pylori, L. monocytogenes, V. parahaemolyticus, Vibrio cholerae,* or *B. cereus*), ova and parasites, and physical characteristics of the urine sample, such as pH and specific gravity. Any analyte can be detected for which a reliable assay can be designed. With reference to the present disclosure the person of ordinary skill in the art will realize a variety of antigens that can be detected using a variety of assay principles applicable in the invention.

Test Kits

[0064] A further aspect of the present invention provides kits containing one or more collection slides of the present invention, and/or one or more assay devices of the present invention, and instructions for their use in carrying out an assay. The test kits can be packaged in a variety of formats, depending upon the needs of the user. In one embodiment the instructions provided with the kit are instructions for detecting the presence of hemoglobin in a stool sample.

[0065] In one embodiment, the kit contains three collection slides, three assay devices, three applicators, a desiccation mailing pouch having three sealable compartments, and instructions for collecting a sample, provided in a package. The package can be any suitable container. In various embodiments the package can be a box, a pouch, a bag, or can be simply a wrapping binding the items of the kit together.

[0066] In another embodiment the kits contain one or more collection slides and assay devices individually packaged in foil pouches, and one or more bottles of extraction buffer, and instructions, provided in a package. In another embodiment the kits contain three individually wrapped collection slides, extraction buffer for performing three tests, and instructions for use. At a health care facility where many tests would be conducted, the kit can contain many individually wrapped test devices, one or two large bottles of extraction buffer, and a single copy of the instructions.

[0067] A further embodiment provides a kit containing two "mini-kits," wherein one mini-kit contains packaged together three collection slides, three applicators, a desiccant mail pouch and instructions for the patient explaining how to correctly collect the samples. The second mini-kit would contain, packaged together for the doctor, three test devices, extraction buffer sufficient to perform three tests and instructions for use.

EXAMPLE 1

Effect of Treatment of Transfer Bead With Surfactant on Buffer Flow Rate

[0068] This example illustrates the benefit of treating the absorbent transfer material with surfactant (Triton® X-100 (synonyms: octyl phenol ethoxylate, polyoxyethylene, Octyl phenyl ether) in manufacturing the assay device.

[0069] Absorbent transfer material in the form of beads was treated with solutions of Tris-casein-PVP buffer containing 0, 1, 2, 3, 4 or 5% Triton® X-100. The saturated transfer beads were then thoroughly dried at 55° C., followed by insertion of each bead into the bead orifice of an assembled test device containing a test strip. Unfilled collection slides having sample pads treated with 0.06 ug Triton® X-100 were engaged with the test devices and 200-240 μl of buffer was applied to each buffer orifice. The time for the control line to appear in the results window was measured. In all cases, the buffer passed through the empty collection slide in 5 seconds. When the transfer bead contained no (0%) Triton® X-100, it took 68 seconds for the control line to appear on the test strips. However, a concentration of 1-5% of Triton® X-100 reduced the time to 19-26 seconds. Therefore, a concentration of 1-5% Triton® X-100 reduces the length of sample flow time substantially.

EXAMPLE 2

Effect of Collection Slide Cover Pad Surfactant Concentration on Buffer Flow Rate

[0070] This example illustrates the benefit of treating the collection slide cover pad with surfactant to obtain a faster flow rate of the buffer.

[0071] All test devices contained transfer beads treated with 1.2 μg of Triton® X-100. The sample collection pads of the collection slides were untreated. The cover pads were treated with 20 μl of 0, 0.31, 0.63, 1.25, 2.5 or 5% Triton® X-100. The empty collection slides were engaged in the test devices and 200 μl of buffer was added to each buffer orifice, to trigger the lateral flow. Buffer was unable to flow into the collection slide when the cover pad was not treated with a surfactant (0% Triton® X-100). As Triton® X-100 concentration increased, the flow rate also increased. When the cover pad was treated with 0.31% Triton® X-100, the control line appeared at 20 seconds. At Triton® X-100 concentrations of 0.63%, 1.25%, 2.5% and 5%, the control

line appeared at 17, 16, 15 and 12 seconds, respectively. However, it was found that at the higher surfactant concentrations (i.e. 1.25% and 5% Triton® X-100) the buffer leaked out of the sample area of the collection slide.

EXPERIMENT 3

Influence of Sample Cover Pad and Transfer Bead on Test Sensitivity

[0072] This example illustrates the ability of the cover pad and sample collection pad to allow the passage of hemoglobin, and therefore not interfere with assay sensitivity.

[0073] Solutions containing 0, 50, 100 and 200 ng hHb/ml were prepared. Collection slides having cover pads treated with 20 μl of 0.53% Triton® X-100 were engaged in the docking area of assay devices of the invention having transfer beads treated with 1.2 μg Triton® X-100. 200 μl of the hHb solutions was applied to the buffer orifices of the collection slides, followed by measurement of the test line intensity at 5 minutes of incubation time. As a control, 140 μl of the hHb solution was applied directly to test strips housed in test devices having no transfer beads. The intensity of the test lines of the control tests was also measured at 5 minutes.

[0074] The test samples and the control samples were found to produce the same results. At a concentration of 0 ng hHb/ml, both the test and control produced negative results. At 50 ng hHB/ml both the devices containing the treated pad/bead and the control device produced a low positive signal. At 100 ng hHb/ml both the devices containing the treated pad/bead and the control device produced a medium positive signal. And at 200 ng hHb/ml both the devices containing the treated pad/bead and the control device produced a medium positive signal. Thus, the cover pad and transfer bead do not retain hHb and have no significant effect on the sensitivity of the test.

EXAMPLE 4

Use of the Collection Slide and Assay Device for Analysis of hBh in Stool

[0075] Three collection slides of the invention are prepared by a patient. At a health care facility, each card is placed into the docking area of an assay device of the invention. By placing the collection slides into the docking area, the hinged side of the slide is inserted under the tang, and the slide pressed downward and snapped into place in the docking area, so that the eluent orifice of the collection slide is in fluid communication with the absorbent transfer material of the device.

[0076] Three drops (about 200 μl) of extraction buffer are applied to the solvent orifice of the collection slide. During a brief (e.g., 5 minutes) incubation period, the buffer is drawn through the sample collection pad and through the absorbent transfer material, and into the test element of the device, where the detection of the analyte (hHb) occurs. The test element is a test strip having at test line with specific binding molecules for hHb, and a reagent zone with labeled antibodies for hHb. After the incubation period has passed, the detection zone of the assay device is observed and found to exhibit both a control line, and a positive result (red line) at the test line, indicating a positive result for hHb in the stool sample.

[0077] The invention illustratively described herein may be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by various embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0078] The contents of the articles, patents, and patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other documents.

1. A device for detecting an analyte in a sample, comprising:

a housing containing

a test element,

a docking area for receiving and engaging an external collection slide, and comprising a sample transfer orifice with an absorbent transfer material disposed therein and in fluid communication with the test element;

a results window for observing a test result.

2. The device of claim 1 wherein the absorbent transfer material is a material selected from the group consisting of: ultra-high molecular weight polyethylene, polyethylene, polyurethane, nylon, polyester, polypropylene, polytetrafluoroethylene, and a cellulose-based material.

3. The device of claim 2 wherein the absorbent transfer material comprises an ultra-high molecular weight polyethylene filter.

4. The device of claim 3 wherein the sample transfer orifice comprises a well in the housing of the device, and the absorbent transfer material is located in the well.

5. The device of claim 3, wherein the absorbent transfer material comprises a surfactant.

6. The device of claim 3, wherein the absorbent transfer material comprises a reagent selected from the group consisting of: polyoxyethylene (23) dodecyl ether, polyoxyethylene (9) lauryl alcohol, poly(oxyethylene-cooxypropylene) block copolymer, p-Isononylphenoxy-poly(glycidol), sorbitol anhydride monostearate, polydimethylsiloxane methylethoxylate, polyethoxylated (20) oleyl alcohol, polyethoxylated (35) castor oil, polyoxyethelene (20) sorbitan monolaurate, polyoxyethelene (20) sorbitan monolaurate, octylphenol ethoxylate (1.2), octylphenoxypolyethoxy (5) ethanol, octylphenoxypolyethoxy (9-10) ethanol, octylphenoxypolyethoxy (30) ethanol, sodium olefin (C14-C16) solfonate, sodium polyoxethylene(1)lauryl sulfate, benzalkonium chloride, ethylenediamine alkoxlate block copolymer,

2,4,7,9-tetramethyl-5-decyne-4,7-diol ethoxylate (10), 2,4,7,9-tetramethyl-5-good wetter decyne-4,7-diol ethoxylate (30), amine alkylbenzene sulfonate, poly(oxyethylene-co-oxypropylene) block copolymer, telomer B monoether, sodium dioctylsulfo-succinate, poly(Vinylmethylether/Maleic Anhydride) copolymer, sodium N-oleyl-N-methyltaurate, dodecylsulfate, sodium taurocholate, sodium cholate, N-cytltrimethylammonium bromide, N,N-dimethyldodecylamine N-oxide, 3-[3-(cholamidopropyl)dimethylammonio]-1-proanesulfonate, alcohol ethoxylate, n-octyl sucrose, n-dodecyl sucrose, n-dodecyl maltoside, octyl glucoside, octyl thioglucoside, n-hexyl glucoside, n-dodecyl glucoside, tris(hydroxymethyl) aminomethane buffer, phosphate buffer, borate buffer, tartrate buffer, phthalate buffer, polyvinylpyrrolidone homopolymer, poly(vinylmethylether/maleic anhydride), polyethylene oxide, polyelthylene glycol, polyvinylalcohol, 1-ethenyl-2-pyrrolidinone, bony fish gelatin, crosslinked polyacrylic acid polymer, hydroxyporpylcellulose, sodium carboxymethylcelluose, sodium polystryenesulfonate, sodium carageenin, acrylic latex, hydroxyethylcellulose, bovine serum albumin, egg white albumin, casein, 5-chloro-2-methyl-isothiazol-3-one and sodium azide.

7. The device of claim 1 wherein

the test element is comprised within the housing and comprises a bibulous matrix having a sample application zone in fluid communication with the absorbent transfer material;

a reagent zone comprising reagents for conducting an assay; and

a detection zone comprising a test line for visually detecting the presence or absence of the analyte at the test line.

8. The device of claim 7, wherein the test line further comprises a specific binding molecule for the analyte immobilized on the matrix.

9. The device of claim 7 wherein the specific binding molecule on the test line binds to human hemoglobin.

10. The device of claim 1 wherein the analyte is human hemoglobin.

11. The device of claim 7 wherein the reagent zone comprises labeled specific binding molecule for the analyte.

12. The device of claim 8 wherein the specific binding molecule is an antibody.

13. The device of claim 1 wherein the docking area further comprises one or more snap locks for holding a sample collection slide in position in the docking area.

14. The device of claim 1 wherein the docking area comprises one or more projections for securing a sample collection slide in position above the absorbent transfer pad.

15. The device of claim 1 wherein the docking area comprises a depression in the housing, which is at least partially circumscribed by a raised area of the housing.

16. A device for collecting and transferring a sample, comprising:

a first card having an inner surface and a eluent orifice;

a second card hingeably connected to the first card and having an inner surface and a solvent orifice, the collection slide having an open position and a closed position, wherein the solvent and eluent orifices are aligned when the collection slide is in the closed position; and

a sample collection area on the first card to which sample is applied for collection.

17. The device of claim 16, wherein the sample collection area further comprises:

a sample collection pad overlaying the eluent orifice on the first card and wherein the sample application area is at least partially circumscribed by a sealing structure on the first card, and;

a cover pad on the second card and overlaying the solvent orifice, wherein the second card further comprises a sealing structure complementary to the structure on the first card.

18. The device of claim 17 wherein the structure on the first card is a gasket, and the structure on the second card is a groove, and the gasket on the first card engages the groove on the second card when the collection slide is in the closed position.

19. The device of claim 17 wherein the cover pad and/or collection pad comprise reagents for eluting analyte from the sample.

20. The device of claim 19, wherein a reagent is selected from the group consisting of: a blocking agent, a surfactant, a wetting agent, a solubilizer, a stabilizer, a diluent and a preservative.

21. The device of claim 19, wherein a reagent is selected from the group consisting of: polyoxyethylene (23) dodecyl ether, polyoxyethylene (9) lauryl alcohol, poly(oxyethylene-cooxypropylene) block copolymer, p-Isononylphenoxy-poly(glycidol), sorbitol anhydride monostearate, polydimethylsiloxane methylethoxylate, polyethoxylated (20) oleyl alcohol, polyethoxylated (35) castor oil, polyoxyethelene (20) sorbitan monolaurate, polyoxyethelene (20) sorbitan monolaurate, octylphenol ethoxylate (1.2), octylphenoxy-polyethoxy (5) ethanol, octylphenoxypolyethoxy (9-10) ethanol, octylphenoxypolyethoxy (30) ethanol, sodium olefin (C14-C16) solfonate, sodium polyoxethylene(1)lauryl sulfate, benzalkonium chloride, ethylenediamine alkoxlate block copolymer, 2,4,7,9-tetramethyl-5-decyne-4,7-diol ethoxylate (10), 2,4,7,9-tetramethyl-5-good wetter decyne-4,7-diol ethoxylate (30), amine alkylbenzene sulfonate, poly(oxyethylene-co-oxypropylene) block copolymer, telomer B monoether, sodium dioctylsulfo-succinate, poly-(Vinylmethylether/Maleic Anhydride) copolymer, sodium N-oleyl-N-methyltaurate, dodecylsulfate, sodium taurocholate, sodium cholate, N-cytltrimethylammonium bromide, N,N-dimethyldodecylamine N-oxide, 3-[3-(cholamidopropyl)dimethylammonio]-1-proanesulfonate, alcohol ethoxylate, n-octyl sucrose, n-dodecyl sucrose, n-dodecyl maltoside, octyl glucoside, octyl thioglucoside, n-hexyl glucoside, n-dodecyl glucoside, tris(hydroxymethyl) aminomethane buffer, phosphate buffer, borate buffer, tartrate buffer, phthalate buffer, polyvinylpyrrolidone homopolymer, poly(vinylmethylether/maleic anhydride), polyethylene oxide, polyelthylene glycol, polyvinylalcohol, 1-ethenyl-2-pyrrolidinone, bony fish gelatin, crosslinked polyacrylic acid polymer, hydroxyporpylcellulose, sodium carboxymethylcelluose, sodium polystryenesulfonate, sodium carageenin, acrylic latex, hydroxyethylcellulose, bovine serum albumin, egg white albumin, casein, 5-chloro-2-methyl-isothiazol-3-one and sodium azide.

22. The device of claim 16 wherein the first card and second card are comprised of plastic.

23. The device of claim 16 wherein the sample is a stool sample.

24. A method of detecting the presence or absence of an analyte in a sample contained in a sample collection slide, comprising:

placing a collection slide containing the sample into a docking area of a device for detecting analyte in a sample, wherein the device comprises:

a test element comprised within a housing;

a docking area for receiving a collection slide and comprising a sample transfer orifice with an absorbent transfer material disposed therein and in fluid communication with the test element; and

a results window for observing a test result; and

wherein the collection slide comprises

a first water resistant card having an eluent orifice;

a second water resistant card hingeably connected to the first card and having a solvent orifice, the collection slide having an open position and a closed position,

a sample collection surface present between the solvent and eluent orifices when the collection slide is in the closed position;

applying an extraction buffer to the solvent orifice of the collection slide;

allowing the extraction buffer to pass through the sample area and into the absorbent transfer bead and test element; and

observing a test result in the results window.

25. The method of claim 24 wherein the test element comprises,

a bibulous matrix comprising

a sample application zone in fluid communication with the transfer bead;

a reagent zone comprising reagents for conducting an assay; and

a detection zone comprising a test line for detecting the presence or absence of the analyte.

26. The method of claim 25 wherein the test line comprises specific binding molecules for the analyte.

27. The method of claim 25 wherein the test line contains reagents for conducting a chemical test.

28. The method of claim 24 wherein the analyte is human hemoglobin.

29. A kit for collecting a biological sample, comprising:

a collection slide comprising:

a first water resistant card having an inner surface and a eluent orifice;

a second water resistant card hingeably connected to the first card and having an inner surface and a solvent orifice, the collection slide having an open position and a closed position, wherein the solvent and eluent orifices are aligned when the collection slide is in the closed position; and

a sample collection area on the first water resistant card to which sample is applied for collection, present between the solvent and eluent orifices when the collection slide is in the closed position; and

a device for detecting an analyte in a fluid comprising:

a housing containing

a test element,

a docking area for engaging a collection slide and comprising a sample transfer orifice, with an absorbent transfer bead disposed therein and in fluid communication with the test element;

a results window for observing a test result;

a sample collector;

an envelope for containing a loaded collection device; and

instructions for use;

provided in a package.

30. A kit according to claim 29 further comprising one or more bottles containing buffers for conducing an assay according to the instructions for use.

* * * * *

Exhibit D

US007488450B2

(12) **United States Patent**
Matusewicz et al.

(10) **Patent No.: US 7,488,450 B2**
(45) **Date of Patent: Feb. 10, 2009**

(54) **ANALYTE COLLECTION AND DETECTION DEVICES**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 731 days.

(21) Appl. No.: **10/794,370**

(22) Filed: **Mar. 4, 2004**

(65) **Prior Publication Data**

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(51) **Int. Cl.**
G01N 31/32 (2006.01)

(52) **U.S. Cl.** **422/58**; 422/50; 422/61; 422/68.1; 422/100

(58) **Field of Classification Search** 422/55–58, 422/68.1, 82.05–82.11; 436/164–172
See application file for complete search history.

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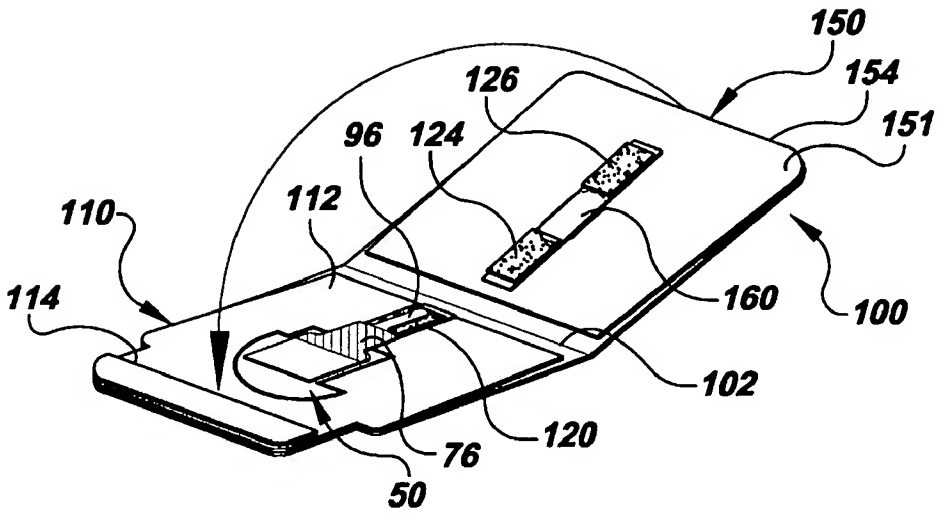
Primary Examiner—Alexa D Neckel
Assistant Examiner—Imran Akram
(74) *Attorney, Agent, or Firm*—Morrison & Foerster LLP

(57) **ABSTRACT**

A system for collecting biological samples, such as fecal specimens, and testing such samples for the presence of an analyte is disclosed. The system comprises a sample collection device that includes a removably secured tab for retaining and transferring a portion of the sample to be tested to the test device. The tab is preferably configured so that it can be received in the test device in only a pre-selected orientation so as to put the portion of the tab carrying the sample in communication with a chromatographic material in the test device.

17 Claims, 7 Drawing Sheets



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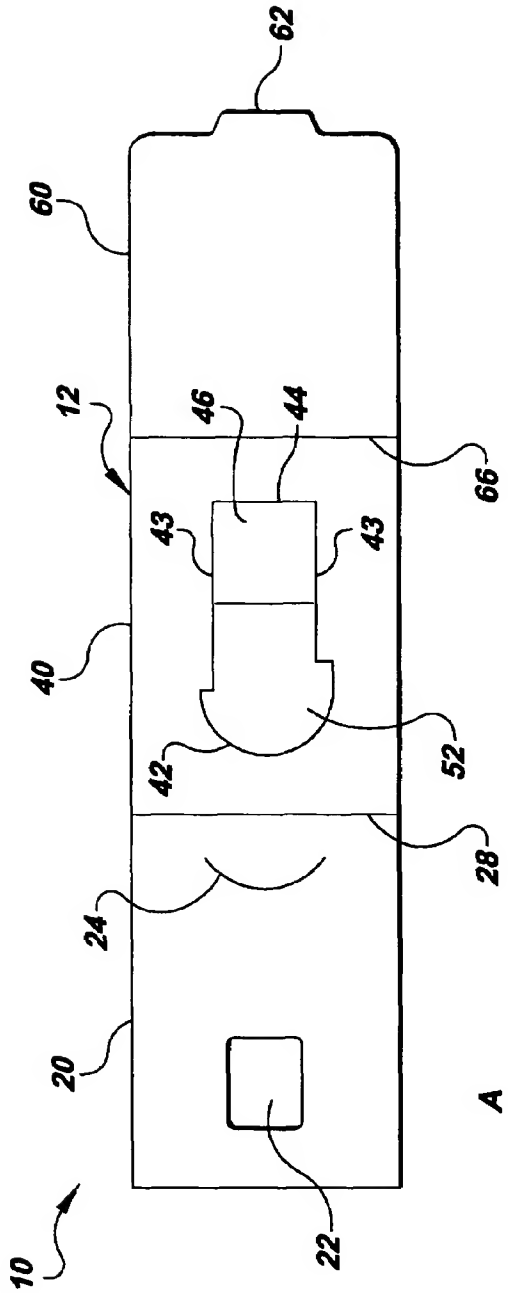
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FIG.1

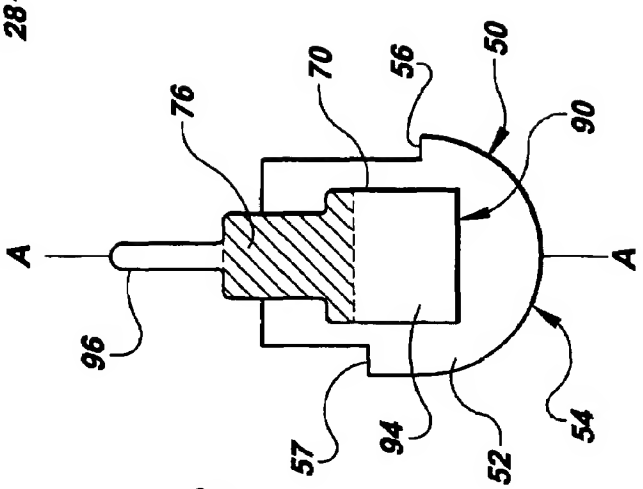
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FIG.2

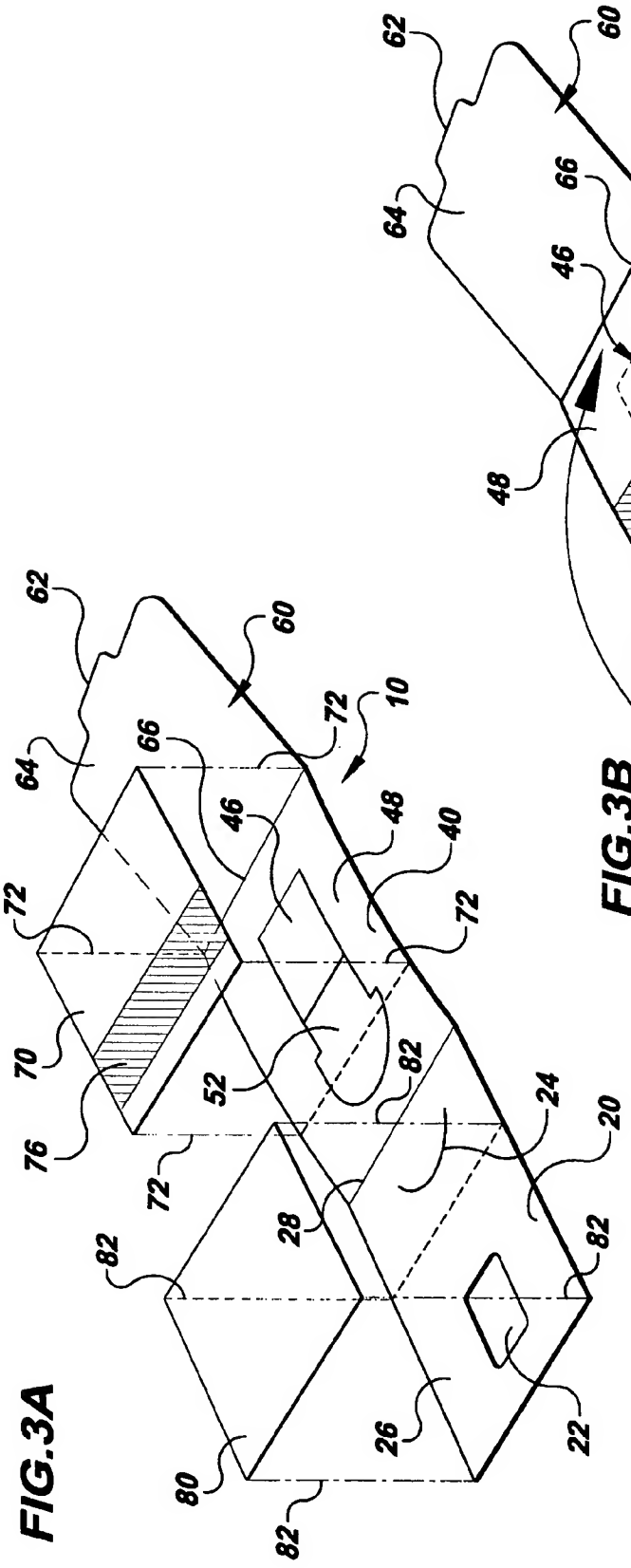
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FIG.3A

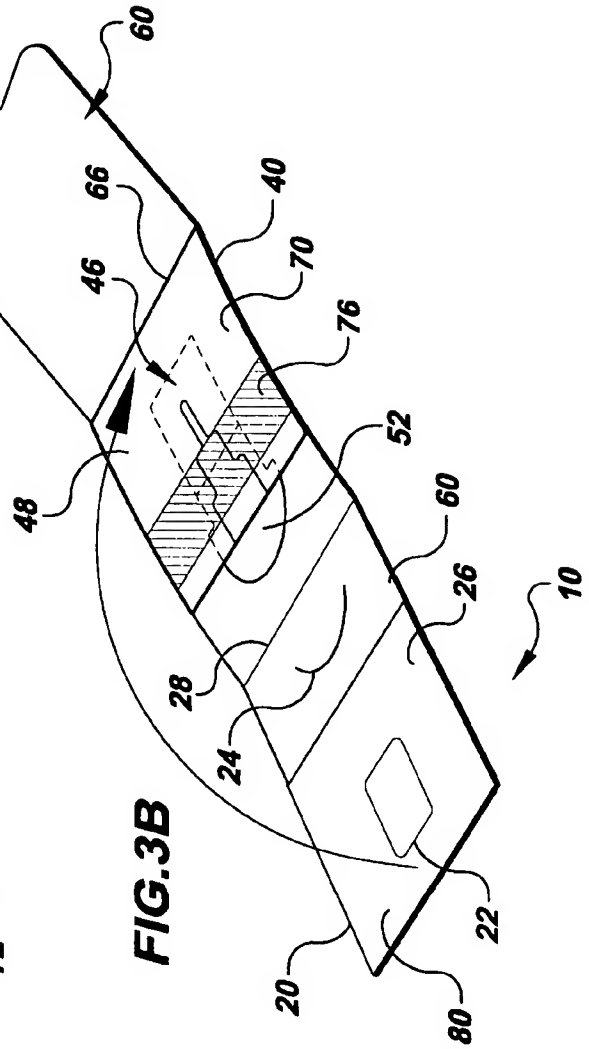
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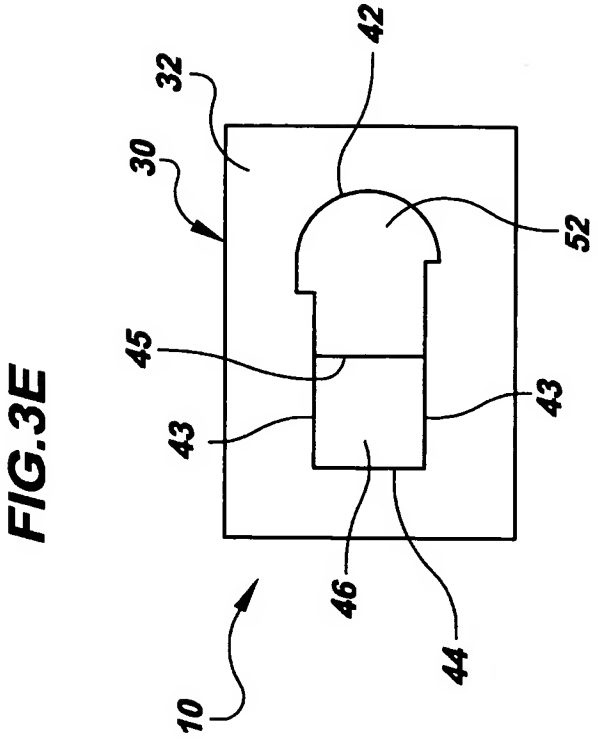
FIG.3B



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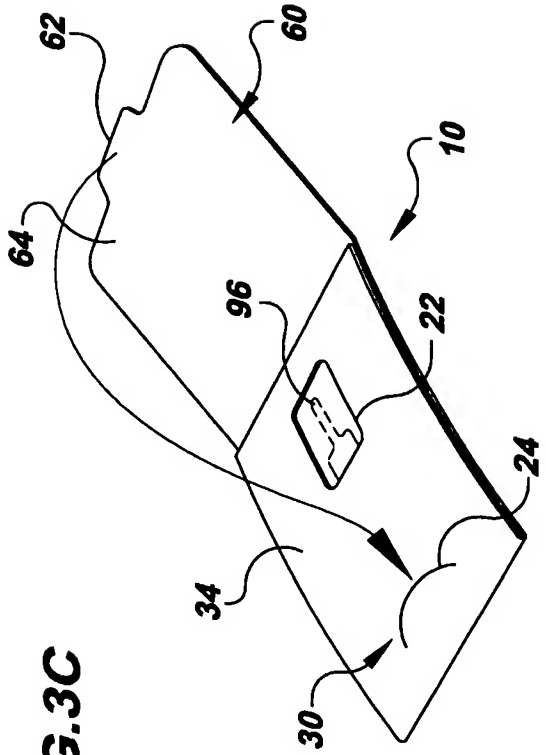

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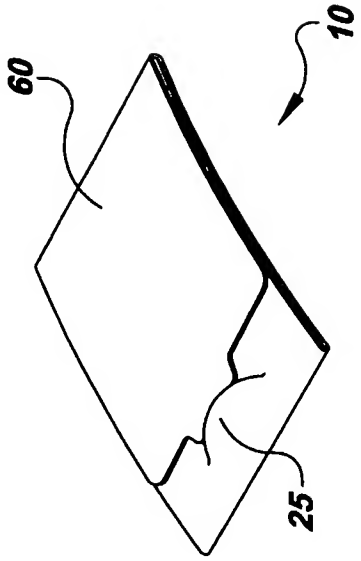

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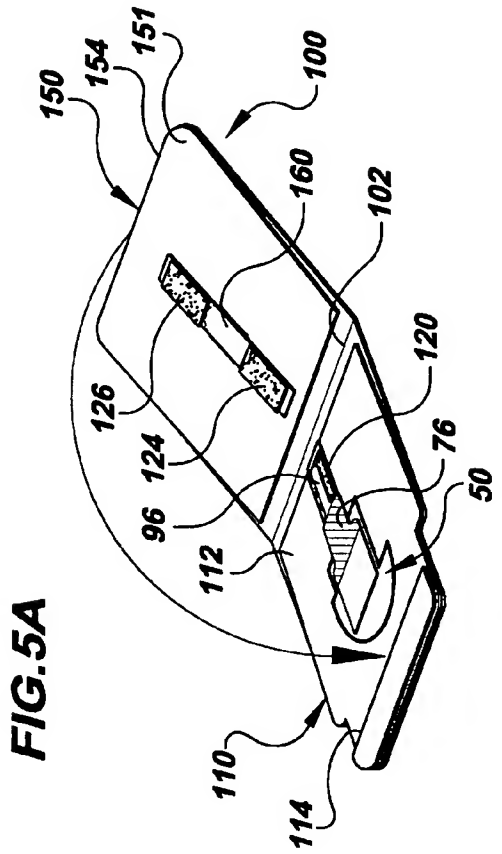

FIG.5A

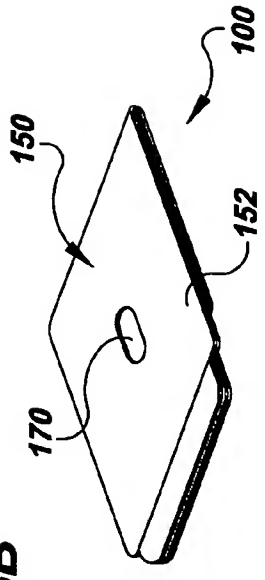

FIG.5B

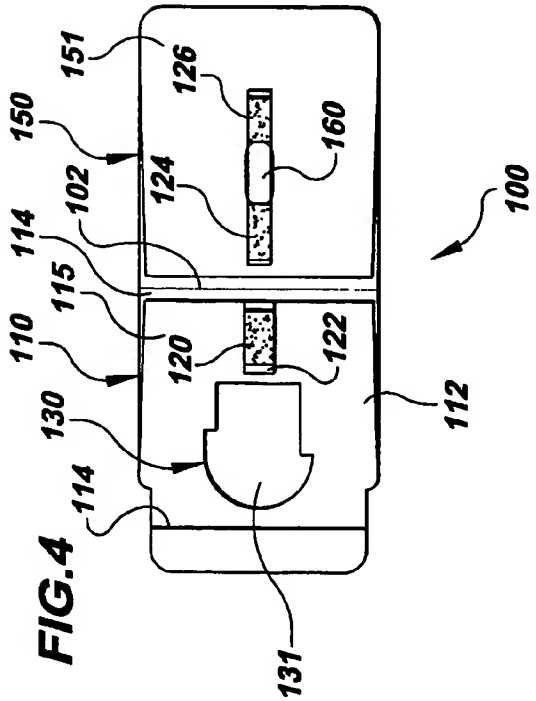

FIG.4

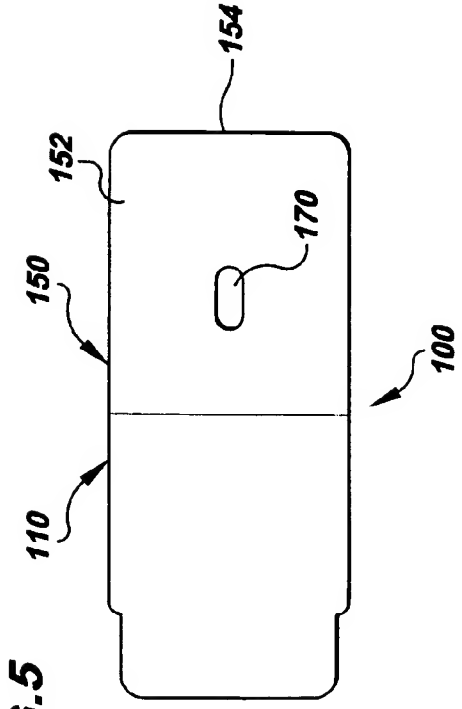

FIG.5

FIG.7

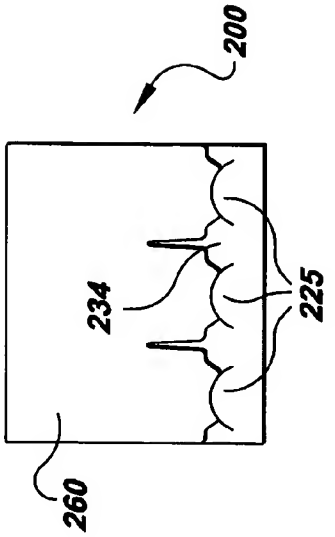

FIG.8

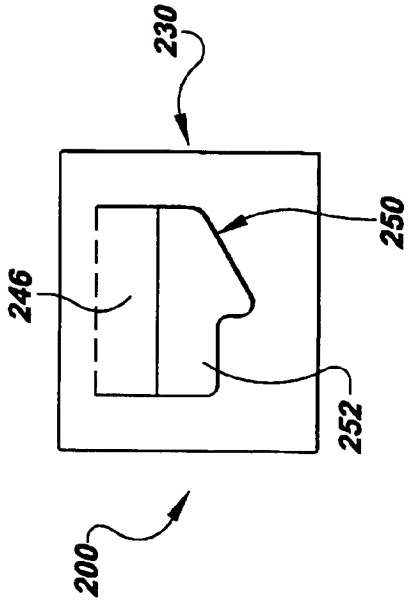

FIG.6

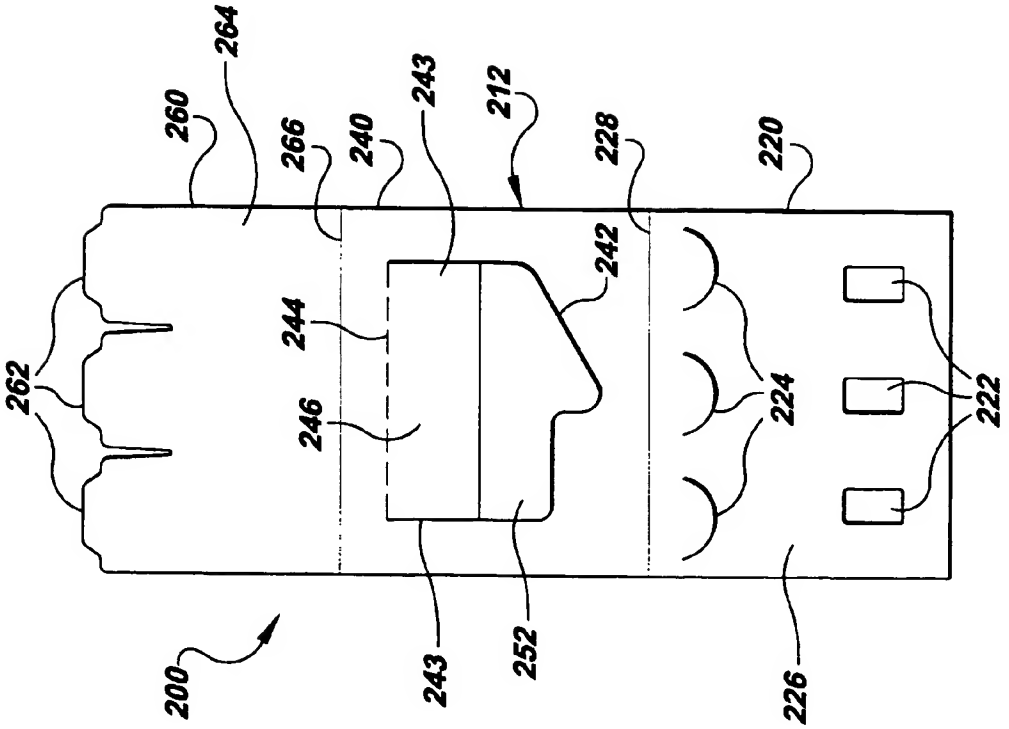

FIG.11

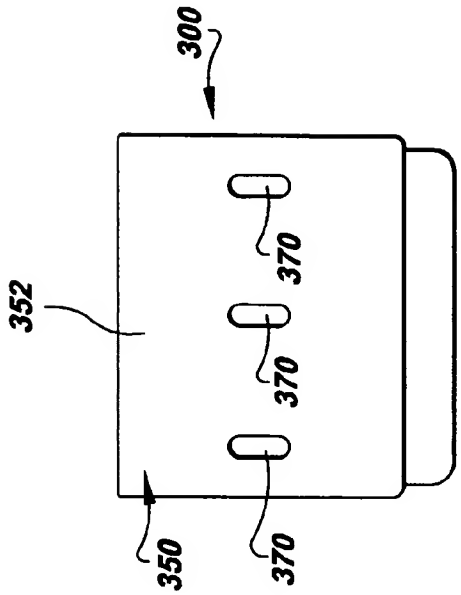

FIG.10

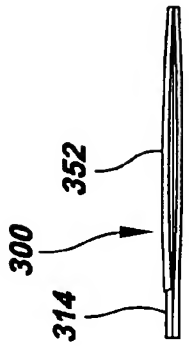

FIG.9

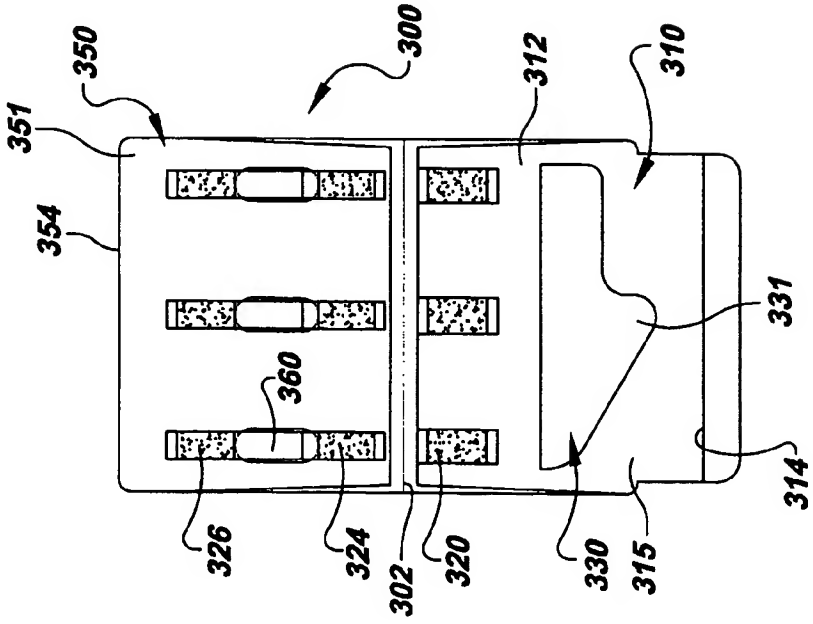

FIG.9B

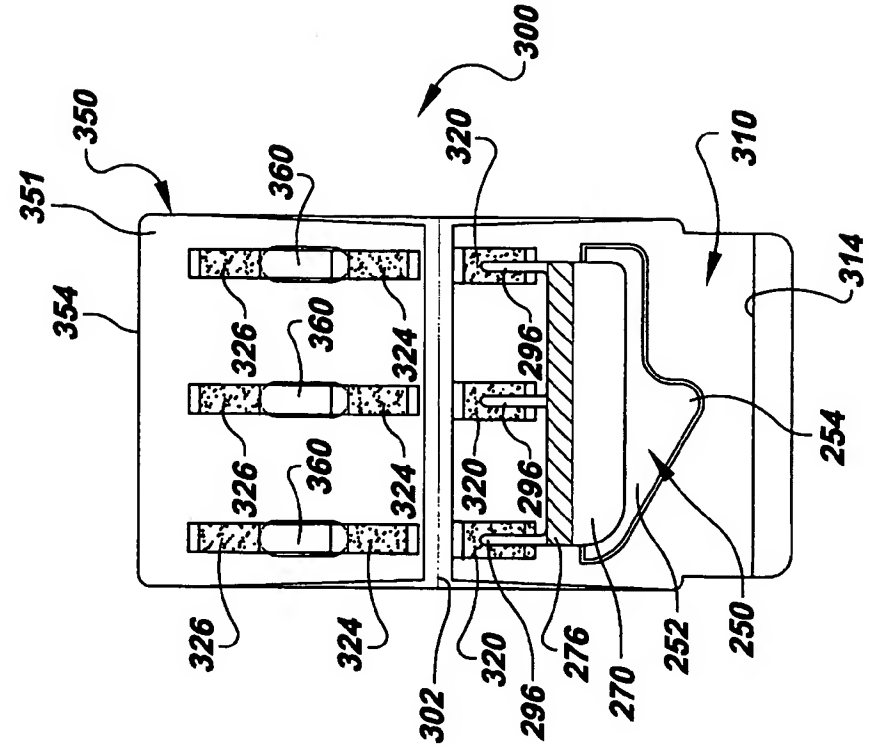
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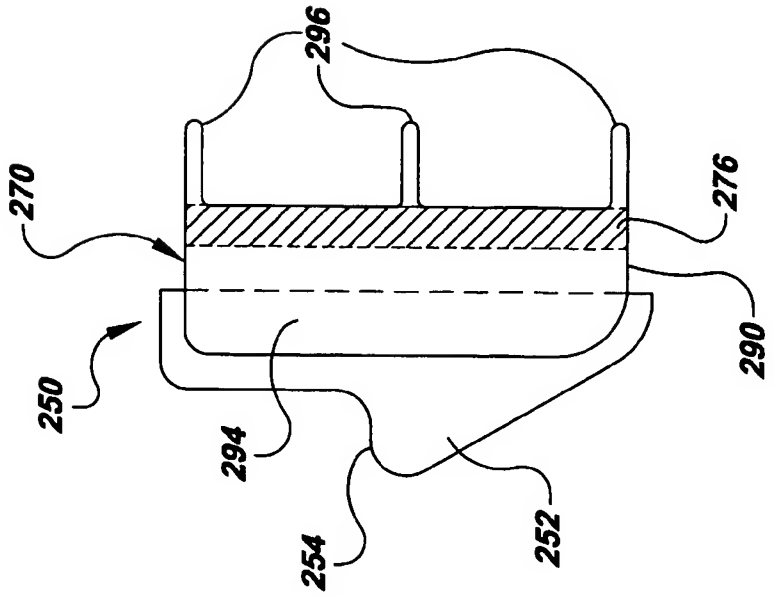
FIG.9A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ANALYTE COLLECTION AND DETECTION DEVICES

BACKGROUND OF THE INVENTION

The present invention is directed to a system for collecting a sample possibly containing an analyte of interest and for then testing the sample. The invention is particularly directed to an improved system for detecting an analyte of interest in a fecal sample. Testing for blood in fecal samples, referred to as a fecal occult blood (FOB) test, for example is commonly performed as a screen for colorectal cancer.

A variety of FOB formats are known in the art (see e.g., U.S. Pat. Nos. 3,996,006; 4,225,557; 4,789,629; 5,064,766; 5,100,619; 5,106,582; 5,171,529; and 5,182,191). The majority of such test formats are based on the chemical detection of heme groups present in stool as a breakdown product of blood. In such tests, the pseudoperoxidase nature of the heme group is used to catalyze a colorimetric reaction between an indicator dye and peroxide. The oxygen sensitive dye can be gum guaiac, orthodianisidine, tetramethylbenzidine, or the like, with guaiac being preferred.

Analytes, including analytes present in stool, can also be detected using chromatographic assay systems. Chromatographic assays, and in particular immunochromatographic assays, are frequently used by physicians and medical technicians for rapid in-office diagnosis and therapeutic monitoring of a variety of conditions and disorders. Immunoassays depend on the specific interaction between an antigen or hapten and a corresponding antibody. In immunochromatographic assays, a detecting reagent or particle is linked to an antibody which binds specifically to a molecule to be assayed, forming a conjugate. This conjugate is then mixed with a specimen and, if the molecule to be assayed is present in the specimen, the detecting reagent-linked antibodies bind to the molecule, thereby giving an indication that the molecule is present. The detecting reagent or particle can be identifiable by color, magnetic properties, specific reactivity with another molecule, or another physical or chemical property.

More information regarding such analytical test systems can be found for example in U.S. Pat. Nos. 4,789,629; 5,441,698; 5,877,028; 6,017,767; 6,165,416; 6,168,956; 6,033,627; 5,846,838; 5,747,351 and 6,221,678, all of which are incorporated herein by reference. In order to make use of such systems, of course, a sample to be tested must first be obtained. In the case of samples obtained from a location away from a physician's office or laboratory able to perform an assay, the sample must be appropriately collected and then transported to the test site.

The collection of fecal samples for testing presents particular challenges, both to the individuals providing such samples as well as to the technicians testing them. Samples obtained away from a testing laboratory must be mailed or otherwise transported, and when received at a laboratory need to be handled without exposing technicians to such samples. The collected samples further need to be appropriately tested. There remains a need for better methods and devices for collecting such samples, transferring the samples to test devices, and performing assays, in particular to facilitate the transfer of a sample to a test device while minimizing the possibility of exposure of a technician to the sample.

SUMMARY OF THE INVENTION

The present invention provides improved devices and methods for collecting, transferring, and testing a sample, in particular a fecal sample for use in diagnosis of a medical condition. One aspect of the invention comprises a sample test device having a test device body, a receptacle in the body adapted to receive a sample carrying member, and a cover member adapted to be placed onto the body. The sample carrying member is bilaterally asymmetrical, and the receptacle is sized and shaped so that the sample carrying member can be received in only a pre-selected orientation. The cover includes a piece of chromatographic material, and the cover member and receptacle cooperate so as to place the chromatographic material in communication with a sample when the sample carrying member is contained in the receptacle in the pre-selected orientation, and when the cover is attached to the body. Preferably, the cover member is attached to the body with a hinge, or alternatively can be adapted to be reversibly attached to the body. The body can be made from an upper and lower layer of material, in which case the receptacle comprises an opening in the upper layer of material. The cover member can further include a window, and the chromatographic material then preferably extends across the window. An immobilized capture reagent which binds an analyte of interest is present in the portion of the chromatographic material located in the window in such an embodiment.

In another aspect, the chromatographic material of the test device is attached to the body of the test device instead of to the cover. The cover in this embodiment is optional, but if used is preferably adapted to be attached to the body, such as with a hinge.

A further aspect of the present invention comprises a sample collection device comprising a body having a substantially planar surface; a sample tab with a handle removably secured to the substantially planar surface, an absorbent material for receiving a sample attached to the sample tab and extending beyond the distal end of the handle, and a flap attached to the substantially planar surface. The flap has a proximal edge covering at least a portion of the absorbent material of the sample tab when the sample tab is removably secured to the first opposed surface. The proximal edge of the flap is adapted to extend away from the substantially planar surface to provide clearance for the absorbent material when the sample tab is removed from the surface. The handle and flap are preferably integrally formed from the substantially planar surface of the collection device. In one embodiment, the absorbent material is impregnated with a band of moisture barrier material separating a sample collection end of the absorbent material from the remainder of the absorbent material. The body of the device also preferably includes a window in a surface opposite the substantially planar surface which is in communication with the absorbent material. The window can further include a filter material which allows liquid to flow through the filter material but which inhibits the flow of solids. A cover over the window is also preferably included.

Another aspect of the present invention comprises a system for collecting and testing a sample that includes a sample collection device and a test device. The sample collection device includes a sample tab for receiving a sample which is removably secured to the sample collection device and has a configuration which is bilaterally asymmetrical. The test device includes a body, a receptacle in the body adapted to receive the sample tab from the sample collection device, and a cover member adapted to be attached to the body which includes a piece of chromatographic material. The receptacle is sized and shaped so that the sample tab can be received in only a pre-selected orientation. The sample tab, cover member and receptacle cooperate so as to place the chromatographic material in communication with the sample tab when

the sample tab is contained in the receptacle in the pre-selected orientation and when the cover member is attached to the test device body.

The sample tab preferably comprises an absorbent material which is impregnated with a band of moisture barrier material separating a sample collection end of the absorbent material from the remainder of the absorbent material in the sample tab. The cover member of the test device also preferably further includes a window having chromatographic material extending across it, and an immobilized capture reagent which binds an analyte of interest is then preferably present in the portion of the chromatographic material located in the window. The body of the test device can further include a sample pad and a cushioning material between the sample pad and the body.

In yet another aspect, the present invention comprises a method of testing for an analyte in a sample. In this method a sample carrying member which is bilaterally asymmetrical and which carries a sample is received by an individual responsible for carrying out the test or assay, and it is placed in a receptacle of a test device in a pre-selected orientation. The receptacle is sized and shaped so that the sample carrying member can be received in the receptacle only in the pre-selected orientation. A test to determine whether the sample contains the analyte is then performed. The test can include adding an assay buffer to the sample carrying member and then placing a cover on the test device, in which case the cover comprises a chromatographic material with a detection reagent, and placing the cover on the test device places the chromatographic material in communication with the sample in the sample carrying member. The test can further include observing a visible indicator of the assay result on the chromatographic material through a window in the cover. In a preferred embodiment, the sample carrying member is removed from a sample collection device to which it is removably secured prior to testing.

BRIEF DESCRIPTION OF THE FIGURES

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

FIG. **1** is a top plan view of a blank from which a sample collection device of the present invention can be made.

FIG. **2** is a top plan view of a sample collection tab according to the present invention.

FIG. **3**A is a perspective view of the blank of FIG. **1** with the placement of two additional sheets of material on this blank.

FIG. **3**B is a perspective view showing the folding of the completed blank of FIG. **3**A along fold line **28**.

FIG. **3**C is a perspective view showing the folding of the blank of FIG. **3**B along fold line **66**.

FIG. **3**D is a perspective view of the assembled sample collection device.

FIG. **3**E is a bottom plan view of the exterior side of the base member of the collection device.

FIG. **4** is a top plan view of the interior side of a test device according to the present invention.

FIG. **5** is a bottom plan view of the exterior side of a test device according to the present invention.

FIG. **5**A is a perspective view of the interior side of the test device shown in FIG. **4** with the sample collection tab placed in the test device, and depicting the closure of the test device in order to perform a test according to the present invention.

FIG. **5**B is a perspective view of a test device of the invention in a closed position.

FIG. **6** is a top plan view of a blank from which an alternative embodiment of the collection device of the present invention can be made.

FIG. **7** is a top plan view of the exterior side of the cover member of the assembled sample collection device of FIG. **6**.

FIG. **8** is a bottom plan view of the exterior side of the base member of the assembled sample collection device of FIG. **6**.

FIG. **9** is a top plan view of an alternative embodiment of the test device of the present invention designed for use with the sample collection device of FIGS. **6-8**.

FIG. **9**A is a top plan view of a sample collection tab for use with the test device of FIG. **9**.

FIG. **9**B is a top plan view of the test device of FIG. **9** with the sample collection tab (shown in FIG. **9**A) placed in the test device.

FIG. **10** is a side elevation view of the test device of FIG. **9** in a closed position.

FIG. **11** is a top plan view of the test device of FIG. **9** in a closed position.

DETAILED DESCRIPTION OF THE INVENTION

FIGS. **1-3**E illustrate the construction of a preferred embodiment of a sample collection device **10** having features of the present invention. A blank **12** is preferably die-cut or otherwise formed from a piece of rigid material as shown in FIG. **1**. The rigid material is preferably a cellulose-based material that is resistant to moisture such as cardboard, paperboard and fiberboard, the surfaces of which have been treated so as to make them moisture resistant, such as through the application of a varnish or laminate material. A preferred material is solid bleached sulfite (SBS) paperboard approximately 0.024 inches thick. Alternatively, the rigid material can be a plastic that is resistant or impervious to moisture such as polypropylene, polyethylene, polystyrene, acrylic, or polycarbonate plastic.

The blank **12** includes a cover member portion **60**, a center portion **40** and a window portion **20**. The center portion **40** and the window portion **20** cooperate to form a base member **30**, as described below. The center portion **40** includes a sample tab perforation **42** around the entire periphery of sample tab backing **52**. Sample tab perforation **42** is not a complete perforation, i.e. it can comprise a line of cuts or holes, or it can comprise a partial cut into the rigid material of the blank **12** (i.e. the cut does not extend entirely through the rigid material), such that sample tab backing **52** remains connected to the remainder of the blank **12** until separated from the sample collection device **10** through the application of pressure or other force to the sample tab backing **52** by a user. It is preferred that the handle end **54** of the sample tab **50** however comprise a complete perforation. Perforations in detachment flap sides **43** can be complete cuts through the rigid material, or like the sample tab perforation can comprise incomplete or partial perforations of the rigid material. Such perforations cooperate with distal flap edge **44** as well as with a portion of the sample tab perforation **42** to form the detachment flap **46**.

The window portion **20** includes an opening or window **22** cut from the blank **12**. The window portion **20** further includes a tab lock perforation **24** used to form tab lock **25** (shown in FIG. **3**D). The tab lock **25** cooperates with a tab **62** on the cover member **60** when the sample collection device **10** is assembled to maintain the sample collection device **10** in a closed position. Tab lock perforation **24** can be a complete

perforation or, like the perforations in detachment flap sides **43**, can comprise either incomplete or partial perforations of the rigid material.

As best shown in FIGS. **3**A-**3**E, a sample collection device **10** can be constructed from the blank **12** through the addition of absorbent material **70** and filter material **80**. The absorbent material **70** and filter material **80** are placed on and adhered to the collection device **10** as shown in FIG. **3**A. Absorbent material **70** is placed on the center portion **40** along placement lines **72** so as to cover the detachment flap **46** and at least a portion of sample tab backing **52**. The filter material **80** is placed on the window portion **20** of the blank **12** so as to cover the window **22**. For ease of manufacturing the absorbent material **70** and filter material **80** can cover the entire width of the blank **12** as shown in the embodiment shown in FIGS. **3**A and **3**B and can be cut from continuous bands of such materials, but the absorbent material **70** and filter material **80** can also cover less area.

The absorbent material **70** is a material which is more absorbent than the material from which the sample tab backing **52** is made and which is able to hold and/or reversibly bind an analyte of interest, but which doesn't react with or permanently bind the analyte under test conditions. The absorbent material **70** can comprise, for example, an open-cell, chemically inert matrix, such as porous plastic, filter paper, glass fiber, or a combination of filter paper and glass fiber, which doesn't bind or react with an analyte of interest. In one embodiment, the absorbent material **70** can comprise a cotton cellulose fiber-based filter paper such as Type 950, manufactured by Ahlstrom Filtration, Inc (Helsinki, Finland). Such materials allow rapid and complete desiccation of a liquid sample carrying an analyte of interest, and minimize the possibility of sample breakdown due, for example, to continued exposure to a liquid environment. The absorbent material can also be pre-impregnated with materials for removing or disguising any odors from, e.g., a fecal specimen.

The filter material **80** is selected to separate any solid portions of a sample from a liquid portion, such that the liquid passes through the filter together with an analyte of interest but the solid portion does not. In one embodiment of the present invention, used to collect biological samples, the porosity of the filter is selected to filter out cellular or particulate matter in samples such as whole blood or fecal specimens. The filter material **80** thus acts as a screen to allow liquid to flow through the window **22** but to inhibit the flow of sample solids through the window **22** and subsequently into the absorbent material **70** of the sample tab **50**. The filter material **80** also preferably should not bind or react with an analyte of interest for which an assay is to be performed with the test device **100**.

Suitable materials that can be used for the filter material **80** include porous plastic, cellulose, paper, nylon, rayon, glass fiber, polyester mesh, and non-woven synthetic fabrics which preferably have tensile strength, are resistant to tearing, and do not bind or react with an analyte of interest. One such material is the type of fine mesh material used for tea bags, such as type HO3249 nonwoven polyester teabag paper manufactured by Ahlstrom Filtration, Inc.

In one embodiment (not illustrated), the absorbent material **70** and filter material **80** are bonded together or otherwise brought into physical contact prior to being attached to the sample collection device **10**, rather than being applied separately to the sample collection device **10**. In this embodiment the absorbent material face of the combined absorbent material **70** and filter material **80** can be adhered first to the center portion **40**, after which the window portion **20** would be bent along fold line **28** to bring the interior side **26** of the window portion **20** into contact with the filter paper **80**. Alternatively the filter paper face of the combined absorbent material **70** and filter material **80** can be adhered first to the interior surface **26** of the window portion **20**, after which the window portion **20** would be bent along fold line **28** to bring the absorbent material **80** into contact with the center portion **40**.

The adhesive is preferably used to adhere the absorbent material and filter material preferably is one that isn't soluble in the sample liquid containing an analyte of interest, and for the absorbent material **70** the adhesive shouldn't be soluble in the reagents applied to the sample collection end **96** in the detection of the analyte. One adhesive that can be used is a composition comprising vinyl acetate and starch such as National Starch adhesive #38-4536. The adhesive can be applied in drops or dollops onto the center portion **40** and the window portion **20** prior to contacting the absorbent material **70** and filter material **80**. Alternatively a band of adhesive material may be applied to center portion **40** and/or the window portion **20**, or the adhesive may instead or in addition be applied to the absorbent material **70** and/or the filter material **80** prior to contacting the sample collection device **10**. With regard to the absorbent material **70** on the center portion **40**, the adhesive should not be placed so as to cause adhesion of the absorbent material to the detachment flap **46**.

As further shown in FIG. **3**B, the absorbent material **70** is impregnated with a band of moisture barrier material **76** so as to form a moisture barrier. The band of moisture barrier material **76** is preferably created prior to the application of the absorbent material **70** to the central portion **40**. The moisture barrier material can for example comprise wax, as disclosed in U.S. Pat. No. 4,983,416 (the contents of which are hereby incorporated by reference). The moisture barrier material can be printed on, i.e. directly transferred from an applicator surface to the absorbent material **70**, or alternatively can be sprayed or otherwise applied with a pump onto the absorbent material **70**. In a preferred embodiment, the moisture barrier material is a water-insoluble polymer such as Fast Drying Polyurethane (available from the Minwax Company). If polyurethane is used, it can be used either straight from the container or diluted with certain organic solvents, e.g., isopropyl alcohol, reagent alcohol (ethanol), heptane, and/or ethyl acetate. Other polymer materials that can be used include polyacrylates and polyvinyl alcohols. Other materials which can be used to form a moisture barrier include those used in the paper industry as sizing materials used for controlling the porosity of paper, such as starch.

FIGS. **3**A and **3**B illustrate an advantageous method of manufacturing a sample tab **50** according to the present invention. The method comprises providing a piece of rigid backing material comprising a substantially planar surface, in this case the blank **12**, and then attaching a piece of absorbent material **70** impregnated with the band of moisture barrier material **76** to the substantially planar surface of the rigid backing material. The portion of the absorbent material **70** comprising the sample collection member **90** is preferably cut or formed from the absorbent material **70** prior to being adhered to the blank **12**. Sample collection member **90** should be adhered to the sample tab backing **52** such that only a base portion **94** (best shown in FIG. **2**) is adhered to the rigid material of the sample tab backing **52** and such that sample collection end **96** is not adhered to the detachment flap **46**.

FIG. **2** shows the sample tab **50** after being removed from the sample collection device **10**. With reference to FIG. **2**, the band of moisture barrier material **76** is interposed between the handle end **54** in a proximal portion of the sample tab **50** and the sample collection end **96** which extends beyond a distal portion of the handle end **54**. The band of moisture barrier

material **76** acts to limit the flow of any liquid introduced to the absorbent material **70** of the sample collection end **96** from flowing into the absorbent material base portion **94**. This allows a larger piece of absorbent material **70** to be used in forming the sample collection end **96**, for ease of manufacturing, but still provides for a more limited and defined area of the sample collection end **96** for retaining an analyte of interest. Although the band **76** is shown in FIG. **3**B as a strip whose outer edges parallel each other, the exact configuration of this band **76** is not important (i.e., it could be shaped as a wave), as long as it serves to limit the flow of any liquid introduced to the absorbent material **70** of the sample collection end **96** into absorbent material **70** outside the sample collection end **96** when the sample tab **50** is tested for an analyte.

As described in further detail below, the sample collection end **96** both absorbs analyte from a sample and transfers such analyte to the test device **100** when a test of such sample is performed according to the present invention. The sample tab **50** can thus also be referred to as a sample carrying member in its capacity of retaining a sample and presenting it for analysis by an appropriate test device. By limiting the absorbance of liquid in the sample collection end **96** to an area of predetermined size, the quantity of analyte absorbed from a sample can be better controlled, resulting in improved assay accuracy and consistency. The use of a different absorbent material **70** may affect the amount of an analyte of interest that such a sample collection end **96** can retain, so the appropriate size of the sample collection end **96** may correspondingly change in different embodiments. The transfer of analyte to the test device **100** can be accomplished more efficiently with this configuration, since a fluid applied to the sample collection end **96** for the purpose of reconstituting the analyte contained in the sample collection end **96** of the sample tab **50** will be confined to the sample collection end **96** and will not substantially flow toward the base portion **94**, which could carry analyte toward such base portion **94** and away from the test device **100**.

As described above, the sample tab backing **52** remains connected to the sample collection device **10** until separated from it through the application of pressure or other force to the sample tab backing **52** by a user. The sample tab **50** can be removably secured to the collection device **10** in other ways as well. For example, in alternative embodiments (not shown) the sample tab **50** can be removably secured by an adhesive applied to a portion of the sample tab backing **52** which overlaps with the collection device **10**, for example the exterior side **32** of the base member **30** (shown in FIG. **3**E), or by a separate piece of material having an adhesive backing which secures the sample tab backing **52** to the remainder of the sample collection device **10**. Thus while the sample tab **50** is secured to the sample collection device **10** during the process of collecting a sample, the sample tab **50** is designed to be removed from the collection device **10** and used with the test device **100** as described in further detail below.

In a preferred embodiment of the present invention, a portion of the sample tab **50**, such as the handle end **54**, comprises a shape which is not bilaterally symmetrical. Handle end **54** can comprise any or all of the structure of sample tab **50** other than the absorbent material **70**. In this embodiment, the receptacle **130** of the test device **100** is sized and shaped so that the sample tab **50** can be received in only a pre-selected orientation. Sample tab **50** thus cooperates with the receptacle **130** in a unique way, such as in a lock-and-key manner. This feature of the present invention ensures that the sample collection end **96** of the sample tab **50** is appropriately directed and is placed in communication with sample pad **120** and ultimately with chromatographic material **160** when it is present in the receptacle **130**.

When a sample liquid or a liquid used in an assay of the present invention (such as a liquid carrying an analyte of interest and/or a detection reagent) is able to pass from one element of the present invention to a second element, whether directly or via an intervening element, the two elements are described as being in communication with each other. Communication generally involves physical contact between the elements (including intervening elements), but physical contact is not required as long as such a liquid is able to travel from one element to the other. Elements of the invention are said herein to be in communication (or in liquid communication) even in the absence of a liquid, as long as such elements cooperate in such a way that when the sample or assay liquid is present it is able to flow from one element to the other.

In the embodiment shown in FIG. **2**, handle end **54** of the sample tab **50** comprises corner elements **56** and **57** which occur on a different horizontal plane with respect to vertical line A-A which runs from the sample collection end **96** through the handle end **54** and thus impart a bilaterally asymmetrical configuration to the handle end **54** of the sample tab **50**. The sample tab **50** of FIG. **2** is likewise bilaterally asymmetrical along an axis perpendicular to line A-A. Bilateral asymmetry of the sample tab **50** as used herein can refer to asymmetry along one or more than one axis.

The assembly of a sample collection device **10** according to the present invention is shown in FIGS. **3**A-**3**E. Once the absorbent material **70** and filter material **80** are secured to the central portion **40** and window portion **20**, respectively, and after the interior side **26** of the window portion **20** is folded toward the interior side **48** of the central portion **40** along fold line **28**, as shown in FIG. **3**B, the interior side **26** of the window portion **20** is secured to the interior side **48** of the central portion **40** to form base member **30** of the sample collection device **10**. The base member **30** comprises a body having opposed surfaces, namely interior side **34** (as seen in FIG. **3**C) and exterior side **32** (seen in FIG. **3**E) of the base member **30**. An adhesive such as that used to adhere the absorbent material **70** and/or the filter material **80** can be used to form the base member **30**. As shown in FIG. **3**C, the sample collection end **96** of the sample tab **50** is in communication with the base member window **22** when the base member **30** has been formed, and while the sample tab **50** remains removably secured to the base member **30**. In this way, when a sample is applied to the window **22**, sample liquid flows through the filter material **80** in the window **22** and then into the absorbent material **70** of the sample collection end **96** of sample tab **50**.

A cover member **60** is preferably included with the sample collection device **10** (though such a cover is not necessary for the successful operation of the sample collection device **10**). As shown in FIG. **3**C, the cover member **60** can be moved into a closed position with respect to the base member **30** by folding the interior side **64** of the cover member **60** toward the interior side **34** of the base member **30** along fold line **66**. The cover member **60** can be joined to the base member **30** by a hinge, such as the crease at the fold line **66**, or alternatively the cover member **60** can be a separate piece (not shown) attached for example by adhesive. The hinge can be an integral part of the sample collection device **10**, as in the illustrated embodiments, or can also be a separate component. The hinge is preferably made of the same material as the sample collection device **10**, but can be a different material compatible with the base member **30** and the cover member **60**.

The cover member **60** is adapted to cover at least the window **22** when in a closed position with respect to the base member **30**, and the interior side **64** of the cover member **60** is also preferably in physical contact with the interior side of the base member. The cover member **60** thus helps to protect someone handling the collection device **10** from coming into contact with a sample placed on the collection device **10** and also protects the sample from contamination and potentially from degradation due to exposure to light or to other elements that might otherwise be able to contact the sample.

The cover member **60** is reversibly secured to the base member **30**. In the embodiment illustrated in FIGS. **3**C and **3**D, cover member **60** is reversibly secured to the base member **30** by means of a tab **62** on the cover member **60** which cooperates with tab lock **25**. In such an embodiment it is advantageous that the cover member be rigid, but it must be capable of flexing in order to be able to position the tab **62** in the tab lock **25**. The cover member **60** can also be reversibly secured to the base member **30** by other means, such as those described below for closing the test device **100**.

The assembled sample collection device **10**, as shown in FIG. **3**D, is preferably substantially planar, as are the cover member **60** and base member **30** components. While other configurations are possible, a planar configuration facilitates delivery of the collection device via mail or courier to a facility which then tests the sample. While the embodiment shown in FIGS. **1**-**3**E is constructed from a single sheet of material which is folded to create two panels, the sample collection device **10** can alternatively be constructed from multiple pieces or layers of material that are laminated or otherwise attached together.

As shown in FIG. **3**E, detachment flap **46** serves to cover the area behind the window **22**, i.e. on the exterior side **32** of the base member **30**. When a liquid-containing sample is applied through the window **22** of the base member **30**, the detachment flap **46** (in cooperation with the sample tab backing **52**, while it is attached to the base member **30**) substantially prevents sample liquid from coming into contact with the exterior side **32** of the base member **30**, or with a user who might be in contact with the exterior side **32**.

One feature of the sample collection device **10** which serves to protect the sample collection end **96** of the sample tab **50** is the design of the detachment flap **46**. The detachment flap **46**, comprising a distal flap edge **44** secured at a distal end to the base member **30** of the sample collection device **10**, further comprises a proximal edge **45** removably secured at a proximal end of the detachment flap **46** to the sample collection device **10** and/or to the distal end of the sample tab handle **54**. The sample tab **50** comprises the sample collection end **96**, which as shown in FIG. **2** comprises a piece of absorbent material **70** extending distally beyond the distal end of the handle **54**. While the sample tab **50** and the proximal edge **45** of the detachment flap **46** are removably secured to the sample collection device **10**, the distal end of the sample tab handle **54** and the proximal edge **45** of the detachment flap **46** are in physical contact or close proximity, and the proximal edge **45** of the detachment flap **46** covers at least a portion of the absorbent material **70** of the sample collection end **96**.

In this embodiment the exterior side **32** of the base member **30** of the sample collection device **10** comprises a rigid material having a substantially planar surface. The detachment flap **46** and sample tab handle **54** are formed from the material of the substantially planar surface, i.e. they comprise an integral part of the same piece of material. The proximal edge **45** of the detachment flap **46** is adapted to detach from the base member **30** and/or from the distal end of the sample tab handle **54**, for example by causing any material bridges along proximal edge **45** joining the detachment flap **46** to the sample tab **50** to become separated. The perforations in detachment flap sides **43** are preferably complete cuts through the exterior side **32** of the base member **30**, but if they comprise incomplete or partial perforations of the exterior side **32** they should be adapted to separate the detachment flap sides **43** from the remainder of the base member **30**.

The separation of the proximal edge **45** and preferably the sides **43** of the detachment flap **46** creates a flap of material (i.e. the detachment flap **46**). The proximal edge **45** of the detachment flap **46** is adapted to extend away from the substantially planar surface to provide clearance for the absorbent material **70** of the sample collection end **96** when the sample tab **50** is removed from the sample collection device **10**. Handle end **54** of the sample tab **50** is adapted to extend away from the base member **30** when base member **30** is flexed convexly (i.e. when the center of the exterior side **32** of the base member **30** rises above the plane formed by the shorter edges of the base member), allowing a user to grip the handle end and pull the sample tab backing **52** from the exterior side **32** of the base member **30**. When the portion of sample tab **50** abutting the proximal edge **45** is detached from and/or moves away from the proximal edge **45**, the detachment flap **46** tends to extend away from the substantially planar exterior surface **32** of the base member **30**, which creates a clearance distance between the sample collection end **96** of the sample tab **50**, which lies under the detachment flap **46**, and the proximal edge **45** of the detachment flap **46**. This clearance allows the sample collection end **96** to be removed from the sample collection device **10** without substantially impacting the structural integrity of the absorbent material **70** of the sample collection end **96**. Without providing such clearance between the sample collection end **96** and the proximal edge **45** of the detachment flap **46**, there is a risk that the sample collection end **96** could be torn or sheared off as it is removed from the sample collection device **10**.

In embodiments where the sample to be collected has an unpleasant odor, the cover member **60** can further include an odor masking agent. For example, the cover member **60** can include a deodorant or perfume, and/or an odor absorbing agent such as baking soda or charcoal.

Once a sample is collected by the sample collection device **10**, it can be tested for an analyte of interest in a test device **100** of the present invention. The test device **100** comprises a base member **110** (also referred to as a test device body) and a cover member **150** having opposed surfaces **151** and **152**. The test device **100** can be made from the same types of materials as the sample collection device **10**, and is preferably made from laminated SBS.

The interior side **112** of the base member **110** includes a receptacle **130** adapted to receive the sample tab **50** of the collection device **10**, preferably the handle portion **54** of the sample tab **50**. The receptacle **130** can comprise for example a depression or cavity in the test device base member **110**. In embodiments in which the test device **100** is molded from a plastic or other material, the receptacle **130** is simply molded to the desired shape to fit or otherwise cooperate with a sample tab **50**. Alternatively, the base member **110** can be constructed from two pieces of material attached together, in which case the base **131** of the receptacle **130** comprises the lower layer **114** of the base member **110** of the test device **100**, while the sides of the receptacle are the inner surface of a portion of the upper layer **115** from which a form has been cut. In the embodiment shown in FIGS. **4** and **5**A the two pieces of material are hingedly connected and formed from a single blank, in this case a four panel blank of which two panels cooperate to form the base member **110** and two other panels

form the cover member **150**. In an alternative embodiment, the receptacle **130** can comprise an opening in the test device base member **110**, i.e. without a lower layer. Such an embodiment would however advantageously include clips or other supports to maintain the sample tab **50** in the receptacle when placed therein. The sample tab **50** can also be optionally maintained in the receptacle **130** by means of an adhesive substance in the receptacle **130**.

The base member **110** of the test device **100** further includes a sample pad **120** for receiving analytes from the sample collection end **96** of the sample tab **50**. The sample pad **120** is preferably constructed from several layers of material. The uppermost layer of the sample pad **120**, which is in direct or at least in fluid contact (i.e., in communication) with the sample collection end **96** of the sample tab **50** when sample tab **50** is positioned in the receptacle **130**, comprises an absorbent sample pad material which is inert, i.e. it does not bind (or does not significantly bind) or react with the analyte of interest. The absorbent sample pad material can be made of any absorbent material that will hold liquid sufficiently so that liquid from a sample (typically a reconstituted sample), including buffers or other assay reagents can be accumulated in the absorbent sample pad material and drawn through the chromatographic medium **160** when it is placed in communication with the chromatographic material **160**. Typical materials for the absorbent sample pad material include, but are not limited to, glass fiber, porous plastic, cellulose, filter paper, and combinations of glass fiber and cellulose. The size and shape of the sample pad **120** can be chosen according to the volume of fluid used in the assay.

Below the sample pad material is preferably a layer of foam or cushioning material (not shown). The cushioning material should be an elastic material which resists compression and tends to spring back to its original conformation. When the cover member **150** is closed over the test device **100** of the present invention and the conjugate pad **124** contacts the sample collection end **96** of the sample tab **50**, the conjugate pad **124** preferably slightly compresses the cushioning material of the sample pad **120**, which causes the cushioning material to exert pressure back on the sample collection end **96**. This serves to maintain the sample collection end **96** in communication with the conjugate pad **124**.

If the rigid material comprising the test device **100** which lies directly below this layer of cushioning material is sufficiently non-absorbent and resistant to water and/or other reagent fluids used to detect the analyte of interest, then no other layers are necessary, and the cushioning layer can be secured to the test device **100**, such as with an adhesive. If the material of the cushioning layer is easily damaged, a stronger piece of material **122**, such as LEXAN™ polycarbonate (available from GE Structured Products, Pittsfield, Mass.), can be bonded to the underside of the cushioning material to give structural strength to the combination and allow it to be attached to the test device **100** during manufacturing without undergoing damage.

The test device **100** further includes a piece of chromatographic material **160** used to detect the presence of an analyte of interest in a sample. In a preferred embodiment, the chromatographic material is attached to the cover member **150**. In this embodiment, a conjugate pad **124** is attached to the cover member **150** and is in communication with the sample pad **120** and or the sample collection end **96** of the sample tab **50** by means of a conjugate ribbon (not shown). The conjugate ribbon, which can be made from a non-woven material such as polyester, is in communication with and preferably overlays the conjugate pad **124** in order to smooth the transfer of assay buffer and reconstituted sample into the conjugate pad **124**. The conjugate pad **124** can generally be constructed in the same way as sample pad **120**, except that it contains assay reagents, as described below. In a preferred embodiment, the conjugate ribbon, conjugate pad **124** and chromatographic material **160** are attached to another piece of material, such as LEXAN™ polycarbonate prior to being attached as a unit to the test device **100**.

When the interior side **151** of the cover member **150** is moved toward the interior side **112** of base member **110** by bending cover member **150** along fold line **102**, and when the test device **100** is thereby closed, conjugate pad **124** is placed in communication with the sample pad **120**, thereby allowing any liquid in the sample pad **120** on the base member **110** (which may contain an analyte of interest) to flow into the conjugate pad **124** on the cover member **150** and then into the chromatographic material **160**.

The chromatographic material **160** in this embodiment is preferably in the opening or window **170**, such that the assay results obtained from performing a test with the chromatographic material **160** can be visualized through the window **170** in the cover member **150**. In this embodiment the piece of the chromatographic material **160** is attached to the cover member **150**, generally to the interior surface **151** of the cover member **150** and/or to the window **170**, and preferably extends across the window **170**. At least a portion of the chromatographic material **160** is visible from the window **170**, i.e. it is within or beneath the window **170**, and this portion should include an immobilized capture reagent which binds an analyte of interest being assayed. In this way the results of a test performed with the chromatographic material **160** can be visualized by a user while the cover **150** of the test device **100** is closed. The window **170** can include a sheet of transparent material such as LEXAN™ polycarbonate interposed between the chromatographic material **160** and the exterior side **152** of the cover member **150**, if the chromatographic material **160** isn't already attached to a transparent material such as polycarbonate. While the use of a window **170** is preferred in the present invention, assay results can also be viewed by opening the cover member **150** and visualizing the chromatographic material **160** after an appropriate amount of time has elapsed to allow the assay to be completed (e.g., 5 minutes), thus making the window **170** an optional feature of the present invention.

In the embodiment shown in FIGS. **4** and **5**A, the chromatographic material **160** is attached to the interior surface **151** of the cover member **150** and is placed across the window **170**. The cover member **150** and receptacle **130** cooperate so as to place the chromatographic material **160** in communication with a sample being assayed when the sample tab **50** is contained in the receptacle **130** in a pre-selected orientation and when the cover member **150** is attached to the body **110** of the test device. In this embodiment the chromatographic material **160** is in communication with an absorbent pad **126** at a second end of the chromatographic material **160**. The absorbent pad **126** absorbs liquid that flows across the chromatographic material **160** and ensures that analytes of interest are wicked across the chromatographic material **160**. The system of the present invention is particularly useful for immunochromatographic assays. The chromatographic material can be any suitable material which can serve as a substrate that can provide an indication of the presence of an analyte of interest, in particular a visible indication. For example, a test strip that can be a liquid-conductive solid phase material to which a detection reagent can be immobilized can be used. It can be a material such as nitrocellulose, to which an appropriately charged detection reagent, such as a monoclonal antibody, can be immobilized. A preferred liq-

uid-conductive solid phase material is a nitrocellulose membrane having a pore size of at least about 1 micron.

Nitrocellulose membranes best adapted for use in connection with immunochromatography of this type have a pore size of about 5-20 microns. The selection of particular pore size dictates the flow rate of the assay. Depending upon the particular application, a faster or slower flow rate may be indicated and an appropriate solid phase material selected. Alternatives to nitrocellulose such as filter paper or a nylon membrane can also be used.

To facilitate handling, it is desirable to provide a backing to the nitrocellulose membrane. A thin plastic sheet stock (e.g., polycarbonate or polystyrene) can be cut to provide a suitable water resistant backing for the chromatographic material **160**. Such sheet stock is selected so as not to interfere with the reading of a test result. For example, the selection of a white or clear sheet stock is generally preferred. In an alternative embodiment, the liquid conductive solid phase material can be sandwiched between such water resistant sheet stock, for example so that information or other indications can be made on the sheet stock.

Samples which can be tested with the system of the present invention include biological fluids such as blood, urine, semen, saliva, or excrement, preferably from a human subject, for the detection and/or diagnosis of disease. Samples from animals, plants, food and water can also be tested. The system of the present invention is particularly useful for the detection of FOB.

The sample collection end **96**, when in contact with the sample pad **120**, is adapted to communicate with the chromatographic material **160** and the conjugate pad **124**. The conjugate pad **124** should be in communication with the sample collection end **96** of the sample tab **50** and with the chromatographic material **160**; and should be located in a liquid path (i.e. in communication) between these two elements. The conjugate pad provides a matrix for the deposition of a labelled detection reagent which is free to migrate when reconstituted. As used herein, the term "reconstituted" shall be used to indicate the placing of a sample, analyte or assay component into a liquid, whether by suspending, solubilizing, rehydrating, or other means, so that the sample, analyte or assay component can be carried by the liquid through components of the test device **100**. Whether a sample and/or an analyte is suspended, dissolved, or otherwise carried in such a liquid will depend on the sample, analyte, and liquid involved. Samples to be analyzed can be applied in liquid or dried form. However, if the analyte of interest is not stable in an aqueous or other liquid environment, it is preferred that the sample be dried in order to preserve such analyte. When testing for the analyte in a desiccated sample, the sample contained in the sample collection end **96** is solubilized, such as through rehydration, and the labelled detection reagent within the conjugate pad **124** is also reconstituted. If analyte is present in the sample, the labelled reagent binds to the analyte and the complex is carried to a detection zone of the chromatographic material **160**.

To perform a sandwich immunoassay, the labelled detection reagent is typically a labeled specific binding partner to the analyte, such as a monoclonal or polyclonal antibody specific for a first epitope of the analyte of interest, coupled to a detectable label. A specific binding partner is a member of a pair of molecules that interact by means of specific non-covalent interactions that depend on the three-dimensional structures of the molecules involved. Typical pairs of specific binding partners include antigen-antibody, hapten-antibody, hormone-receptor, nucleic acid (e.g., DNA or RNA) strand-complementary nucleic acid strand, substrate-enzyme, inhibitor-enzyme, carbohydrate-lectin, biotin-avidin, and virus-cellular receptor. Antibodies can include both intact antibody molecules of the appropriate specificity and antibody fragments as well as chemically modified intact antibody molecules and antibody fragments.

The detectable label can be coupled to an antibody or other specific binding partner by any of the applicable techniques known in the art including, for example, covalent bonding and passive adsorption. The detectable label is typically mobile, in that it can migrate through the chromatographic medium **160** with the antibody or other specific binding partner, whether free or bound to an analyte.

The detectable label may be a direct or an indirect label. A direct label is a label which is readily visible in its natural state, either to the naked eye, or with the aid of optical devices. A label which is visible only in the presence of external stimulation, such as ultraviolet light, is also considered to be a direct label. Examples of direct labels include dye sols (e.g., colloidal carbon), metallic sols (e.g., colloidal gold, silver, or iron), fluorescent particles and colored latex particles. A preferred metallic label is colloidal gold, while a preferred non-metallic colloidal label is colloidal carbon. Colloidal carbon labels for labeling of specific binding partners are described, for example, in U.S. Pat. No. 5,529,901 to Van Doorn et al., while colloidal gold labels are described in U.S. Pat. No. 6,528,323, both incorporated by this reference. Antibodies labeled with colloidal gold are commercially available, for example from Sigma Chemical Company, St. Louis, Mo.

Indirect labels require the addition of one or more developing reagents, such as substrates, to facilitate detection. Such labels include enzymes such as alkaline phosphatase and horseradish peroxidase.

In order to conduct an immunoassay with the chromatographic material **160**, an immobilized capture reagent should also be included in the chromatographic material **160**. The immobilized capture reagent is also typically a monoclonal or polyclonal antibody which is specific for a second epitope or range of epitopes on the analyte of interest. Alternatively, when one of the specific binding partners is labeled with biotin, the secondary specific binding partner (i.e., the immunochromatographic capture reagent) can comprise a molecule conjugated to avidin. Thus, analyte present in the sample, whether bound by the detection reagent or not, is bound by the immobilized binding reagent in the detection zone of the chromatographic material **160**. If a direct label is employed, a visible line appears on the chromatographic material **160** as bound label accumulates in the detection zone. The appearance of this line may be diagnostic for the presence of analyte of interest in the sample.

A control zone can also be integrated into the chromatographic material, **160**. The function of a control zone is to convey a signal to the user which indicates only that the testing process is complete and that the binding interaction which results in the detectable signal (unrelated to detecting the analyte of interest) has taken place as expected. For example, if the detection reagent is a murine monoclonal antibody linked to a detectable label, then the control zone can comprise an "anti-mouse" polyclonal antibody immobilized to the liquid-conductive solid phase material, preferably downstream of the detection zone. At least some of the detection reagent not bound in the detection zone through a sandwich interaction involving the analyte of interest should ultimately bind in the control zone.

The test device cover member **150**, test device base member **110**, and sample tab **50** are adapted to cooperate so that the sample collection end **96** of the sample tab **50** is in liquid communication with the chromatographic material **160** when

the handle portion **54** of the sample tab **50** is contained in the receptacle **130** of the test device base member **110** and when the test device cover member **150** is reversibly attached to the test device base member **110**. In this embodiment the results of an assay performed with the test device **100** are visible through the window **170** when the cover member **150** is closed, and the bound portion of the detection reagent is present on the portion of the piece of chromatographic material **160** that is in the window **170**. In an alternative embodiment, in which the chromatographic material **160** is attached to the base member **110**, the portion of the chromatographic material **160** showing the assay results can be viewed without the cover member **150** being placed over or attached to the base member **110**, or a window can be provided in the cover member **150** to allow viewing of the assay results through such window when the cover member **150** is attached to the base member **110**.

The test device **100** is preferably reversibly secured in a closed position with a closure capable of maintaining sample collection end **96** in communication with the sample pad **120** and conjugate pad **124** when the test device **100** is closed. A variety of closures can be used, such as for example a strip of adhesive on the interior side **151** of the cover member **150** and/or on the interior side **112** of the base member **110**. In another embodiment, such a closure, appropriate for use when the rigid material of the test device **100** is SBS, can comprise a bevel as shown in FIGS. **5**A and **5**B. The bevel closure of the testing device according to the present invention can be the bevel closure disclosed in U.S. Pat. No. 5,441,698 of Norell, incorporated herein by this reference. To form a bevel closure, a bevel edge **114** is created on the base member **110**. Typically, the bevel angle is between about 5 degrees and about 30 degrees from the vertical, and more preferably the angle is from about 8 degrees to about 10 degrees from the vertical. The cover edge **154** is undercut, and the bevel angle and the undercut angle are approximately equal.

To close the test device **100** using such a bevel closure, the cover member **150** is rotated about fold line **102** (a hinge) such that the cover member **150** and base member **110** are brought together with a protruding corner of the cover edge **154** brought against the upper surface of the bevel edge **114**. The cover member **150** and base member **110** are urged together, slightly flexing the test device **100**, allowing the protruding corner of the cover edge **154** to be displaced beneath an overhanging of the bevel edge **114** on the base member **110**. With the entire length of the cover edge **154** thus captured by the overhanging corner of the bevel edge **114** (shown in FIG. **5**B), the test device **100** is closed.

In a further embodiment of the present invention (not shown), the chromatographic material **160** is located on the base member **110**, i.e. on substantially the same planar surface as the receptacle **130** and the sample pad **120**, and is in liquid communication with the sample pad **120**. As with other embodiments, this test device **100** comprises a receptacle **130** that is adapted to cooperate with a sample tab **50** of the sample collection device **10** of the present invention which is not bilaterally symmetrical, so that the sample collection end **96** of the sample tab **50** is placed in communication with the chromatographic material **160** when the sample tab **50** (and in particular the handle end **54** of the sample tab **50**) is received in the receptacle **130**. If a cover member **150** is used with this embodiment, the cover member preferably reversibly securable to the base member **110** and includes a window **170** through which the results of the test performed on the chromatographic material can be visualized.

In another embodiment of the present invention, the system of the present invention comprises a sample collection device **10** and test device **100** as described above, except that the sample tab **50** and the receptacle **130** with which it cooperates to perform an assay according to the invention do not have an asymmetrical configuration. In this embodiment, the sample tab **50** is removably secured to the base member **30** of the sample collection device **10** as described previously. The cover member **150** of the test device **100** in this embodiment preferably includes a window **170** which provides communication between the interior side **151** and the exterior side **152** of the cover member **150**. A portion of the piece of chromatographic material **160** attached to the cover member **150** (preferably to the interior side **151** of the cover member **150**) is in the window and the assay results are visible in the portion of the piece of chromatographic material **160** within the window **170** by a user of the test device. In this embodiment the test device cover member **150**, test device base member **110**, and sample tab **50** are still adapted to cooperate so that the sample collection end **96** of the sample tab **50** is in communication with the chromatographic material **160** when the handle portion **54** of the sample tab **50** is contained in the receptacle **130** of the test device base member **110** and when the test device cover member **150** is removably secured to the test device base member **110**.

Both the sample collection device **10** and the test device **100** according to the present invention are preferably single-use devices, meant to be disposed of and not reused after the collection of a sample with the sample collection device **10** and/or after the performance of an assay with the test device **100**. In an alternative embodiment, portions of the test device **100** can be used more than once. As will be clear to one of skill in the art, the sample collection pad **120**, absorbent ribbon, absorbent pad **126**, conjugate pad **124** and the chromatographic material **160** of the test device **100** should not be reused, so in an embodiment of the invention in which part of the test device **100** is reused the sample collection pad **120**, absorbent ribbon, absorbent pad **126**, conjugate pad **124**, and chromatographic material **160** should be removably or reversibly secured to the test device **100**. Following the performance of an assay, the used sample collection pad **120**, absorbent ribbon, absorbent pad **126**, conjugate pad **124**, and chromatographic material **160** are removed and replaced with an unused sample collection pad **120**, absorbent ribbon, absorbent pad **126**, conjugate pad **124**, and chromatographic material with which a subsequent assay can be performed.

EXAMPLE 1

Construction of a Sample Collection Device

A sample collection device **10** as shown in FIGS. **1**-**3**E is constructed as follows. A blank **12** approximately 9¼ inches long and 2¼ inches wide made of SBS is die-cut as shown in FIG. **1**. The blank **12** includes a cover member portion **60**, a center portion **40** and a window portion **20**. The center portion **40** includes a sample tab perforation **42** around the entire periphery of sample tab backing **52**. Sample tab perforation **42** is not a complete perforation, such that sample tab backing **52** remains connected to the remainder of the blank **12**. Such perforations cooperate with collection distal flap edge **44** as well as with a portion of the sample tab perforation **42** to form the detachment flap **46**.

The window portion **20** includes an opening or window **22** cut from the blank **12**. The window portion **20** further includes a tab lock perforation **24** used to form tab lock **25** (shown in FIG. **3**D).

FIG. 3A shows the spatial placement of an absorbent material **70** along placement lines **72** and filter material **80** along placement lines **82**. A band of moisture barrier material **76** is applied to the absorbent material **70**, and the absorbent material **70** is also pre-cut to form sample collection member **90**, both prior to the absorbent material **70** being adhered to the filter material **80**. The combined sheet of absorbent material **70** adhered to the filter material **80** is itself then adhered to the window portion **20** with drops of adhesive placed approximately ¼ inch from the edges of the combined sheet (including on the base portion **94** of the sample collection member **90**). The combined sheet is placed on the window portion **20** of the blank **12** so as to cover the window **22**.

The interior side **26** of the window portion **20** is then folded along line **28** until it is brought into contact with and adhered to the interior side of center portion **40** to form the base member **30**. The placement of the combined sheet of absorbent material **70** and filter material **80** on the center portion **40** is such that the detachment flap **46** and at least a portion of sample tab backing **52** is covered.

The cover of the device is next formed by folding cover member portion **60** along line **66** such that the interior surface **64** of the cover member **60** is brought toward the interior surface **34** of base member **30**. The tab lock **25** cooperates with a tab **62** on the cover member **60** to maintain the sample collection device **10** in a closed position.

EXAMPLE 2

Construction of a Test Device

A sample test device as shown in FIGS. **4-5**B is constructed as follows. The test device **100** comprises a base member **110** (approximately 3 inches long and 2½ inches wide) and a cover member **150** (approximately 2½ inches long and 2½ inches wide) connected by a hinge (fold line **102**). The interior side **112** of the base member **110** includes a receptacle **130** adapted to receive the handle end **54** of the sample tab **50** of the collection device **10**. In the embodiment shown in FIGS. **4** and **5**A the base member **110** is constructed from two pieces of material attached (folded) together, and the base **131** of the receptacle **130** comprises the lower layer **114** of the base member **110** of the test device **100**. The sides of the receptacle are the inner surface of a portion of the upper layer **115** from which a form has been cut.

A sample pad **120** for receiving analytes from the sample collection end **96** of the sample tab **50** is attached to the base member **110** of the test device **100**. Receptacle **130** is adapted to receive the sample tab **50** (shown in FIG. **5**A) in only one way, thereby assuring that the sample collection end **96** of the sample tab **50** is placed in contact with sample pad **120**. The handle end **54** of the sample tab **50** and corresponding receptacle are formed with a shape which is not bilaterally symmetrical to accomplish this. Below the sample pad is a layer of foam or cushioning material attached to a strip of LEXAN™ polycarbonate.

A piece of LEXAN™ polycarbonate to which has been attached a conjugate ribbon in contact with a conjugate pad **124**, and which is in further contact with a strip of chromatographic material **160** is also attached to the cover member **150**. The chromatographic material **160** is positioned in an opening or window **170** in the cover member **150** so that results obtained from performing a test with the chromatographic material **160** can be visualized through the window **170**. The piece of LEXAN™ polycarbonate is interposed between the chromatographic material **160** and the exterior side **152** of the cover member **150**.

The conjugate pad **124** and chromatographic material **160** are further positioned on the cover member **150** such that when the cover member **150** is closed over the base member **110** by means of the hinge, the conjugate pad **124** contacts the sample collection end **96** and the sample pad **120**, and the conjugate pad **124** slightly depresses the cushioning material of the sample pad **120**. A absorbent pad **126** is placed in contact with a second end of the chromatographic material **160**. The absorbent pad **126** absorbs liquid that flows across the chromatographic material **160** during an assay.

To close the test device **100**, the cover member **150** is rotated about fold line **102** such that the interior side **151** of the cover member **150** and the interior side **112** of the base member **110** are brought together with a protruding corner of the cover edge **154** brought against the upper surface of the bevel edge **114**. The cover member **150** and base member **110** are urged together, slightly flexing the test device **100**, allowing the protruding corner of the cover edge **154** to be displaced beneath an overhanging of the bevel edge **114** on the base member **110**. With the entire length of the cover edge **154** thus captured by the overhanging corner of the bevel edge **114** (shown in FIG. **5**B), the test device **100** is closed.

The sample collection device and test device of the present invention have been described above in embodiments in which only one sample is tested for the presence of an analyte. However, in other embodiments a single device can collect or analyze more than one sample. If the sample collection device is being used to collect fecal samples, multiple samples can be collected over a predetermined period of time. For example, three different samples can be collected over, e.g., the course of three days with the embodiments described below. Collecting and then analyzing a plurality of samples makes it more likely that an analyte of interest, such as blood in the stool, if present, will be detected, as blood may not be present in a patient's stool in every sample, or the portion of the stool in which blood can be found is not sampled for detection. When performing assays with a test device according to this aspect of the invention, individual samples can be tested one or more at a time, but multiple samples are preferably assayed at the same time with this embodiment.

In describing this alternative embodiment of the present invention, like figure numbers (e.g., **12** and **212**) will denote similar device elements. Elements with the same name (though a different assigned figure number) will also generally be made in the same way and with the same materials unless otherwise noted.

EXAMPLE 3

Construction of a Sample Collection Device for Collecting Multiple Samples

A sample collection device as shown in FIGS. **6-8** is constructed as follows. A blank **212** approximately 9⅛ inches long and 3⅞ inches wide made of SBS is die-cut as shown in FIG. **6**. The blank **212** includes a cover member portion **260**, a center portion **240** and a window portion **220**. The center portion **240** includes a sample tab perforation **242** around the entire periphery of sample tab backing **252**. Sample tab perforation **242** is not a complete perforation, such that sample tab backing **252** remains connected to the remainder of the blank **212**. Such perforations cooperate with collection distal flap edge **244** and detachment flap sides **243** as well as with a portion of the sample tab perforation **242** to form the detachment flap **246**.

The window portion **220** includes an opening or window **222** cut from the blank **212**. The window portion **220** further includes a tab lock perforation **224** used to form tab lock **225** (shown in FIG. **7**).

A band of moisture barrier material **276** (seen in FIG. **9**A) is applied to the absorbent material **270**, and the absorbent material **270** is also pre-cut to form sample collection member **290**, both prior to the absorbent material **270** being adhered to the filter material (not shown). The combined sheet of absorbent material **270** adhered to the filter material is itself then adhered to the window portion **220** with drops of adhesive placed approximately ¼ inch from the edges of the combined sheet (including on the base portion **294** of the sample collection member **290**). The combined sheet is placed on the window portion **220** of the blank **212** so as to cover the window **222**.

The interior side **226** of the window portion **220** is then folded along line **228** until it is brought into contact with and adhered to (with the glue beads) the interior side of center portion **24**Q to form the base member **230**. The placement of the combined sheet of absorbent material **270** and filter material on the center portion **240** is such that the detachment flap **246** and at least a portion of sample tab backing **252** is covered.

The cover of the device is next formed by folding cover member portion **260** along line **266** such that the interior surface **264** of the cover member **260** is brought toward the interior surface **234** of base member **230**. Each of the tab locks **225** cooperate with a corresponding tab **262** on the cover member **260** to maintain the sample collection device **200** in a closed position.

EXAMPLE 4

Construction of a Test Device for Collecting Multiple Samples

A test device for testing three samples as shown in FIGS. **9-11** is constructed as follows. The test device **300** comprises a base member **310** (approximately 3 inches long and 3¼ inches wide) and a cover member **350** (approximately 2⅞ inches long and 3¼ inches wide) connected by a hinge (fold line **302**). The interior side **312** of the base member **310** includes a receptacle **330** adapted to receive the handle end **254** of the sample tab **250** of the collection device **200**. In the embodiment shown in FIGS. **9** and **9**B the base member **310** is constructed from two pieces of material attached together, and the base **331** of the receptacle **330** comprises the lower layer **314** of the base member **310** of the test device **300**. The sides of the receptacle are the inner surface of a portion of the upper layer **315** from which a form has been cut.

Sample pads **320** for receiving analytes from the sample collection ends **296** of the sample tab **250** are attached to the base member **310** of the test device **300**. Receptacle **330** is adapted to receive the sample tab **250** (shown in FIG. **9**B) in only one way, thereby assuring that the sample collection ends **296** of the sample tab **250** are each placed in contact with one of the sample pads **320**. The handle end **254** of the sample tab **250** and corresponding receptacle **330** are formed with a shape which is not bilaterally symmetrical to accomplish this. Below each sample pad **320** is a layer of foam or cushioning material.

On the cover member **350** are placed conjugate pads **324** in contact with strips of chromatographic material **360** also attached to the cover member **350**. The strips of chromatographic material **360** are positioned in windows **370** in the cover member **350** so that results obtained from performing tests with the strips of chromatographic material **360** can be visualized through the windows **370**. Sheets of a clear polymer material such as LEXAN™ polycarbonate are interposed between the chromatographic material **360** and the exterior side **352** of the cover member **350**.

The conjugate pads **324** and strips of chromatographic material **360** are further positioned on the cover member **350** such that when the cover member **350** is closed over the base member **310** by means of the hinge, the conjugate pads **324** contact the sample collection ends **296** and the sample pads **320**, and the conjugate pads **324** slightly depress the cushioning material of each of the sample pads **320**. Further sample pads **326** are placed in contact with a second end of each of the strips of chromatographic material **360**. The further sample pads **326** absorb liquid that flows across the strips of chromatographic material **360** during an assay.

To close the test device **300**, the cover member **350** is rotated about fold line **302** such that the interior side **351** of the cover member **350** and the interior side **312** of the base member **310** are brought together with a protruding corner of the cover edge **354** brought against the upper surface of the bevel edge **314**. The cover member **350** and base member **310** are urged together, slightly flexing the test device **300**, allowing the protruding corner of the cover edge **354** to be displaced beneath an overhanging of the bevel edge **314** on the base member **310**. With the entire length of the cover edge **354** thus captured by the overhanging corner of the bevel edge **314** (shown in FIGS. **10** and **11**), the test device **300** is closed.

In the embodiment of the test device **300** described in Example 4, the test device **300** can alternatively comprise a molded, hard "clamshell" which contains the test device elements. A multiple-assay configuration of the present invention will in most cases be wider than a single assay embodiment, and such a wider configuration may cause contact between a sample collection end **296** of the sample tab **250** and a sample pad **320** to be greater for the assays run on the outer edges of the device as compared with assay(s) performed on the interior strips of chromatographic material **360** if the material of test device **300** is not sufficiently rigid (as may be the case if SBS is used). This can result in a faster chromatographic flow rate on the outer chromatographic strips and cause inaccurate test results. As an alternative to a hard plastic clamshell, a less rigid material like SBS can be used together with strips of more rigid material (not shown) in the base member **310** and cover member **350** to provide additional support. In embodiments using a hard clamshell, the clamshell may be reusable, though certain elements of the test device **300** (described above) should not be reused.

EXAMPLE 5

Collecting a Fecal Specimen for Testing

A fecal sample may be collected in ways known to the art. A sample is preferably obtained which is free of interfering substances, and which is not contaminated by matter from another individual. In one method, a patient from whom a sample is to be collected makes use of a toilet to collect a sample. The toilet (free of toilet bowl cleaners and other chemicals) is first flushed. The lid and seat of the toilet are lifted, and a piece of plastic wrap is placed over the toilet bowl. The plastic wrap can be about two feet long and about a foot wide. The plastic wrap should be secured across the rim of the toilet bowl, preferably across the back half, allowing the middle of the plastic wrap to hang down just above the water. A piece of tissue is then preferably placed on top of the plastic wrap. The patient then lowers and sits on the seat and

has a bowel movement, thereby placing stool on the tissue. Alternatively, the tissue can be used without the plastic wrap. In this case the tissue is placed in the toilet bowl and allowed to float on the surface of the water. The tissue should cover enough of the surface of the water that the stool is placed on the tissue as a result of a bowel movement by the patient. It is important to keep the stool sample from contacting the water in a toilet bowl to the extent possible, as certain analytes, including blood in stool, may only be present on the surface of a stool sample and such analytes are subject to being washed away from the stool sample should they come into contact with water, which could lead then to a false negative test result.

Once the stool sample is obtained, it is applied to the window **22** in communication with the sample collection end **96** of the sample tab **50**. Using a flattened stick or other applicator, a pea-sized sample of stool is collected and placed on the filter paper **80** on one side of the window **22** and spread across half of the window. The applicator is commonly made from wood or can be made from a substantially non-absorbent inert material.

A second pea-sized sample is next preferably collected from a second area of the stool sample, placed on the filter paper **80** on the other side of the window **22**, and spread across the remaining half of the window. The stool samples applied to the filter paper **80** are preferably then mixed so that the sample collection end **96** comes into contact with material from both samples from the stool sample. In one embodiment an application area for the first and second samples can be printed on the absorbent material **70** (or alternatively on the filter paper **80**) in order to guide the user as to where to apply the samples.

The sample is next allowed to dry. A cover may be placed over the sample tab window, as long as it does not seal the window and prevent drying. The collection device should not be placed in a bag or other container that would prevent drying during this time. In embodiments with multiple collection windows, stool samples may be collected over multiple days in the same manner. The collection device is then sent, such as via mail or courier, to a testing facility.

EXAMPLE 6

Performing an Assay with the Test Device

To perform a test for an analyte of interest in a sample collected as described above, the sample tab backing **52** or at least a portion thereof is separated from the exterior side **32** of the base member **30** of the sample collection device **10**, for example by flexing the base member **30** of the device, so that the handle end **54** of the sample tab can be gripped by a user. The sample tab **50** is then carefully removed from the sample collection device, such as by pulling the sample tab **50** up and away from the exterior side **32** of the base member **30**. As described above, at least the proximal edge **45** of the detachment flap **46** also separates from the exterior side **32** to provide clearance for the sample collection end **96** of the sample tab as it is removed from the sample collection device **10**.

The sample tab or sample carrying member **50**, and in particular the handle portion **54** of the sample tab **50** is next placed in the receptacle **130** of the test device **100** as shown in FIG. **5**A. Sample tab **50** is bilaterally asymmetrical and is sized and shaped so that the it can be received in the receptacle **130** only in a pre-selected orientation which ensures that the sample collection end **96** is placed in communication with the sample pad **120** of the test device **100**.

A test for an analyte of interest is next performed with the test device **100**. An appropriate buffer for the analyte to be tested for is next placed on the sample pad **120** and sample collection end **96** of the sample tab **50** in order to reconstitute the analyte, which is typically dry or desiccated. To test for fecal occult blood **3** drops of sample extraction buffer are applied. The sample extraction buffer will be different for different analytes as well as for different antibodies or other detection reagents. For a fecal specimen being assayed for the presence of blood, the extraction buffer preferably comprises an aqueous solution at controlled pH which includes salts, surfactant, and phosphate buffered saline. The antibody preferably binds an epitope on a human globin chain.

The cover member **150** is then placed on the test device, and a chromatographic material comprising a detection reagent is thereby placed in communication with the sample in the sample tab. Reconstituted sample flows from the sample collection end **96** of the sample tab **50** (and from the sample pad, if any sample has migrated into the sample pad after the application of the buffer) into the conjugate pad **124**. The cover member **150** is then closed and assay results read through window **170** after 5 minutes.

Although the present invention has been described in considerable detail with reference to certain preferred versions thereof, other versions are possible. For example, the collector and test component need not be rectangular, but can be in other configurations such as an oval, circular, and polygons having more than four sides. Therefore, the spirit and scope of the appended claims should not be limited to the description of the preferred versions contained herein.

All features disclosed in the specification, including the claims, abstracts, and drawings, and all the steps in any method or process disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive. Each feature disclosed in the specification, including the claims, abstract, and drawings, can be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise, each feature disclosed is one example only of a generic series of equivalent or similar features.

What is claimed is:

1. A sample collection device comprising:

(a) a body having first and second opposed surfaces, wherein the first opposed surface is substantially planar;

(b) sample tab removably secured to the first opposed surface, comprising a handle having a proximal end and a distal end;

(c) an absorbent material for receiving a fecal sample, the absorbent material being attached to the distal end of the sample tab; and

(d) a flap connected to the first opposed surface, the flap comprising a proximal edge covering at least a portion of the absorbent material of the sample tab when the sample tab is removably secured to the first opposed surface;

wherein the proximal edge of the flap is adapted to extend away from the first opposed surface when the first opposed surface is flexed convexly to provide clearance for the absorbent material when the sample tab is removed from the first opposed surface.

2. The sample collection device of claim **1**, wherein the first opposed surface of the body comprises a piece of rigid material, and wherein the sample tab comprises a sample tab backing formed by perforations in the piece of rigid material, the sample tab backing comprising the handle.

3. The sample collection device of claim 1, wherein the flap is formed in the first opposed surface of the body.

4. The sample collection device of claim 1, wherein the absorbent material is impregnated with a band of moisture barrier material separating a sample collection end of the absorbent material from the remainder of the absorbent material.

5. The sample collection device of claim 1, wherein a portion of the absorbent material extends beyond the distal end of the handle.

6. The sample collection device of claim 1, wherein the second opposed surface comprises a window in liquid communication with the absorbent material.

7. The sample collection device of claim 6, wherein the window includes a filter material which allows liquid to flow through the filter material but which inhibits the flow of solids.

8. The sample collection device of claim 6, further including a cover member adapted to cover the window of the second opposed surface.

9. The sample collection device of claim 1, wherein the body of the sample collection device is formed from a cellulose-based material resistant to moisture.

10. A method of collecting a fecal sample, comprising:

(a) providing a sample collection device comprising:

(i) a rigid body having a substantially planar surface;

(ii) a sample tab removably secured to the substantially planar surface of the rigid body, the sample tab comprising a handle having a proximal end and a distal end attached to an absorbent material which carries a fecal sample; and

(iii) a flap having a distal end attached to the substantially planar surface of the rigid body and a proximal end abutting the distal end of the handle;

(b) flexing the rigid body, thereby detaching the proximal end of the handle from the substantially planar surface and causing the proximal end of the flap to extend away from the substantially planar surface; and

(c) pulling the handle away from the flap.

11. A device for the collection and transportation of a fecal sample comprising:

(a) a substantially planar body having front and back opposed surfaces, wherein said front surface includes a sample collection area configured to receive the sample;

(b) a sample collection member removably secured to the back surface of the substantially planar body, wherein a region of the sample collection member is in fluid communication with the sample collection area, wherein said sample collection member is configured to remove a portion of the sample received by the sample collection area when separated from the back surface of the substantially planar body; and

(c) a flap attached to the back surface of the substantially planar body, wherein said flap includes a proximal edge that covers the sample collection area and covers at least a portion of the sample collection member when the sample collection member is removably secured to the back surface.

12. The device of claim 11, wherein the removably secured sample collection member comprises a handle end configured to extend away from the back surface of the substantially planar body when the substantially planar body is flexed, thereby facilitating the detachment of the sample collection member from the back surface of the substantially planar body.

13. The device of claim 11, wherein the flap is configured to extend away from the back surface of the substantially planar body when the sample collection member is detached from the substantially planar body.

14. The device of claim 11, wherein the sample collection member is asymmetrical, wherein the sample collection member is sized and shaped to be received in a pre-selected orientation in a test device.

15. The device of claim 11, further comprising a filter material interposed between the sample collection area and the sample collection member, wherein the filter material allows the flow of liquid and analyte of interest through the filter material to the sample collection member but inhibits the flow of solids.

16. The device of claim 11, further comprising a cover member attached to the front surface, the cover member adapted to cover the sample collection area.

17. The device of claim 11, wherein the sample collection member comprises an area of predetermined size that is in fluid communication with the fecal sample, to remove a substantially consistent quantity of the fecal sample received by the sample collection area when separated from the back surface of the substantially planar body.

* * * * *

Exhibit E

US006077711A

United States Patent [19]

Singer

[11] Patent Number: 6,077,711

[45] Date of Patent: Jun. 20, 2000

[54] **FRANGIBLE AMPULE SPECIMEN TEST CARD**

[76] Inventor: **Jason Singer**, 2331 N. Southport, Chicago, Ill. 60614

[21] Appl. No.: **09/030,174**

[22] Filed: **Feb. 25, 1998**

[51] **Int. Cl.**[7] **G01N 33/72**

[52] **U.S. Cl.** **436/66**; 436/164; 436/165; 436/169; 422/55; 422/56; 422/58

[58] **Field of Search** 422/55, 56, 58; 436/66, 164, 165, 169

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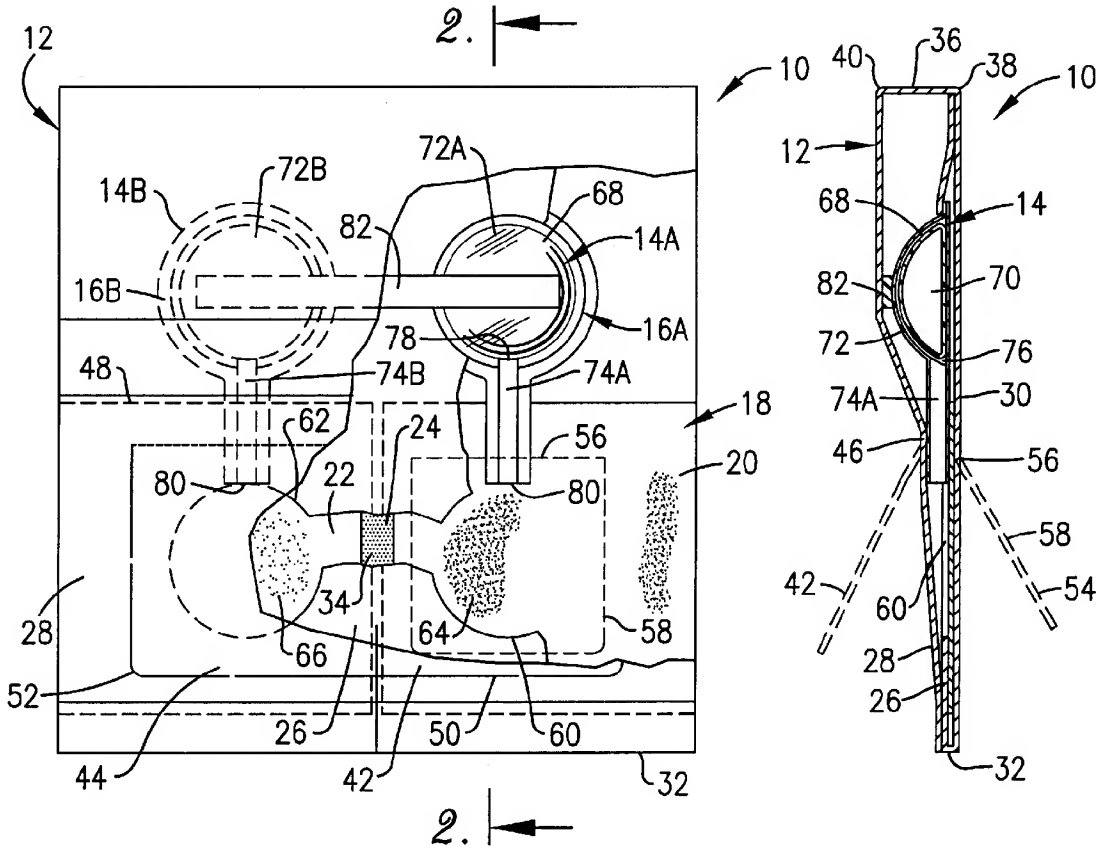
Primary Examiner—Terrence R. Till
Assistant Examiner—Jennifer McNeil
Attorney, Agent, or Firm—Hovey, Williams, Timmons & Collins

[57] **ABSTRACT**

A method and apparatus for testing tissue samples is provided which includes an integrated specimen test card. The test card is especially useful in testing for the presence of blood in stool samples and includes a frangible ampule contained within a channel mounted on the test card, the ampule containing a developer and the test slide being impregnated with a chromatographic reagent. Upon crushing the ampule within the channel, the developer is directed along a canal to a test section of the slide where, upon diffusion of the developer through the slide and moistening of the test slide by the developer in the presence of blood, a chromatographic reaction occurs to indicate to the user the presence of blood. A discrete control section having a second slide impregnated with reagent and a control indicator, such as hemoglobin, may be provided so that developing liquid can be directed simultaneously both to the test section of the slide and the control section of the slide.

35 Claims, 1 Drawing Sheet



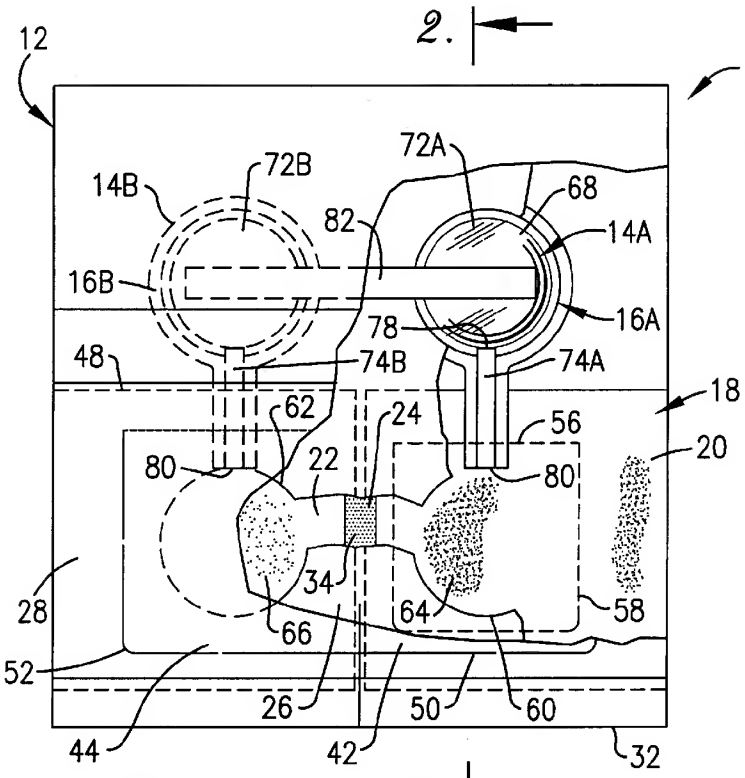


Fig. 1.

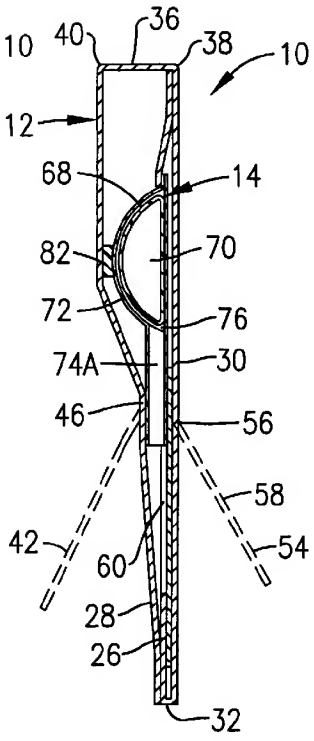


Fig. 2.

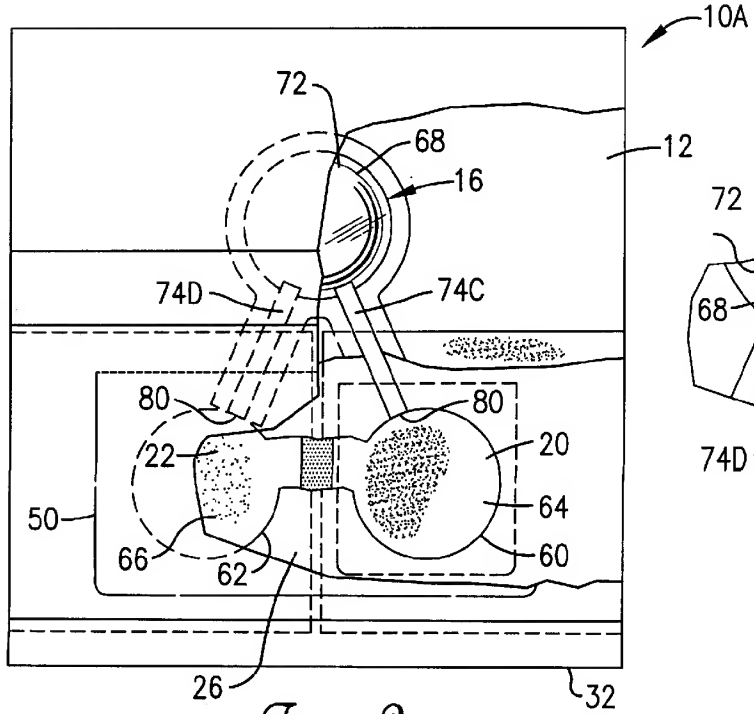


Fig. 3.

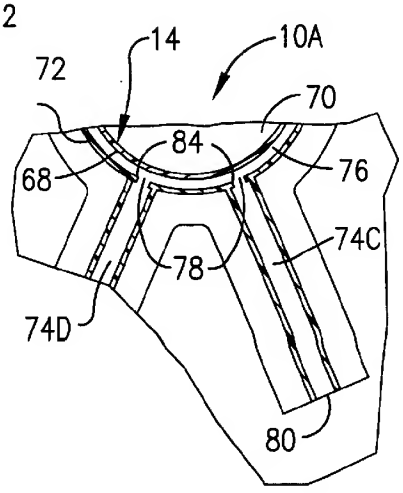


Fig. 4.

FRANGIBLE AMPULE SPECIMEN TEST CARD

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a test medium for medical specimens such as stools which is self-contained including the developer for permitting single use without the need for additional articles for administering the developer. More particularly, it is concerned with a test card which includes a frangible ampule containing a developer and located within a channel. Upon the application of pressure to break the ampule, the developer flows within the channel and is directed to a specimen receiving surface or slide having a reagent thereon for permitting quick and easy handling and testing of the specimen.

2. Description of the Prior Art

In the field of medical diagnostics, it is desirable to obtain rapid test results, preferably on-site without the need for referring the sample to a laboratory. Such tests are known in the medical arts, for example, in occult blood test slide cards for determining the presence of blood in feces, also known as the stool. One existing test card for determining the presence of blood in the stool is sold by SmithKline Diagnostics, Inc. under the trademark Hemoccult, and further illustrated and described in U.S. Pat. No. 4,365,970, with specific slides and their compositions further described in U.S. Pat. Nos. 4,329,317 and 4,382,064 incorporated herein by reference.

The aforementioned test card employs a pivotal cover over the reagent carrying test slide which is beneficial, but requires separate handling of a bottle containing the developing solution to be applied to the slide with the specimen received thereon. As a result, a separate bottle of developing solution is required for use with the test card. This produces problems in that the developing solution is frequently misplaced, the additional time required to use the bottle, and the fact that the feces smeared card must be manipulated and the flap controlled, all while unscrewing the bottle cover and applying the developing solution. In addition, the control region of the card is remotely located from the specimen-receiving test slide such that further applications of the developer are required to a separate area. As a result of the need to handle the separate bottle, the application of the developer to the control region may be infrequent. An improved test card is needed which provides both a control and a test slide but which avoids the problems set forth above.

SUMMARY OF THE INVENTION

These and other objects are largely met by the frangible ampule specimen test card of the present invention. That is to say, the test card as shown and described herein permits the user to apply the sample to the test slide of the card and direct a flow of developer from a frangible ampule carried on the card through channels leading to the slide. Moreover, the frangible specimen test card hereof permits the developer to flow both to the slide carrying the specimen as well as to a control slide without the necessity for carrying a separate bottle which requires manipulation and may be misplaced. Beneficially, the preferred embodiment of the test slide hereof is configured to inhibit application of the specimen to the control region and to thereby enhance proper usage.

Broadly speaking, the frangible specimen test card hereof includes a carrier carrying a specimen-receiving test slide

onto which a medical specimen is placed for testing, a frangible ampule containing a quantity of developer, and a channel for directing a flow of the developer directly to the test slide. A separate control slide impregnated with a chromatographic reagent may be provided, with either a separate frangible ampule and channel fluidically communicating with the control sample or, alternatively, the same frangible ampule being provided with a channel system which divides the flow of developer and directs it to both the sample slide and the control slide. The slides are made of material which is fluid permeable and the developer comes into contact with substantially all of each slide through capillary action or direct fluid flow or a combination thereof. The slides are separated by the carrier which is of cardboard, synthetic resin or less permeable material and which is sufficiently resistant to fluid flow to provide independent test results of the sample and control slides and act as a barrier therebetween. In preferred embodiments, the carrier is glued together between center and back panels, the glue acting as a barrier to flow of the developer between the test slide and the control slide.

In a first alternate embodiment, the channel may be divided into two canals each provided with check valves for inhibiting backflow of any developer from one slide to another. In another embodiment where two separate frangible ampules are provided, a rigid member may be placed in spanning relationship over channels containing the two frangible ampules whereby the user may press the member to break both ampules simultaneously.

Other advantages and details of the invention will be readily appreciated by those skilled in the art with reference to the drawings and the detailed description thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a plan view of a first embodiment of the frangible ampule specimen test card hereof, with a one ampule, channel and a portion of one slide shown in phantom and portions of the carrier and front hinged flap broken away for clarity to show the test slide and the rear flap in phantom;

FIG. **2** is a side elevational view thereof showing the hinged flaps over the front and rear portions of the carrier lifted for access to the front of the slides and the rear portion of the test slide;

FIG. **3** is a plan view of an alternate embodiment of the frangible specimen test card hereof with a portion of the carrier broken away, showing the use of a single frangible ampule contained within the channel and two separate canals leading to two different slides; and

FIG. **4** is an enlarged cross-sectional view of the frangible ampule and channel shown in FIG. **3**, showing the use of check valves to restrict the flow of developer to inhibit backflow from one canal to the other.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring now to the drawing, a frangible ampule specimen test card **10** is shown in FIGS. **1** and **2** and broadly includes a carrier **12**, a frangible ampule **14**, a channel **16**, and at least one slide **18** for receiving a specimen such as a stool specimen thereon. The test card **10** as described herein is particularly useful in determining the presence of occult blood in a stool specimen. Advantageously, the slide **18** may be divided into a segregated first slide section **20** for receiving a tissue specimen to be tested and second slide

section **22** which may be impregnated with a chromatographic reagent for serving as a control to verify the developer within the ampule is operative, with a barrier **24** provided between the first and second slide sections to inhibit any communication of fluid therebetween. Also, as shown in U.S. Pat. No. 4,365,970, incorporated herein by reference, separate positive and negative control areas may be defined by an additional tab (shown as reference character "34" in the U.S. Pat. No. 4,365,970) presenting two holes therein for receiving developer applied thereon for providing a control of either a positive or negative result.

In greater detail, the carrier **12** may be formed of thin cardboard or other protective and substantially non-permeable material such as synthetic resin and preferably, presents a center web **26**, a front side **28** and a back side **30**. The back side **30** is shown connected to the center web **26** by a bottom fold **32** and also by a line of glue **34** which serves both as barrier **24** and also retains the first slide section **20** and second slide section **22** in position. Other relatively impermeable materials may be used as barrier **24**, or one of the center web **26** or back side **30** could include an extension which divides the first and second slide sections. The front side **28** is joined to back side **30** by panel **36** along back fold **38** and front fold **40**, and further may be glued or otherwise secured such as by a staple along the bottom edge of the front side **28**.

The front side **28** includes a pair of front flaps **42** and **44** positioned over the first slide section **20** and the second slide section **22** respectively, each of the flaps **42** and **44** being pivotally carried by respective front hinges **46** and **48**. It may be understood that a single front flap extending over both the first and second slide sections may be provided instead of separate flaps. The other boundaries of the front flaps **42** and **44** other than the hinges are defined by U-shaped front flap margins **50** and **52** which may be either a line of weakening such as perforations, a line of separation formed by die cutting, or by a free margin when the front flaps **42** and **44** extend downwardly co-extensive with the remainder of the front side **28** as viewed in FIG. **1**.

Similarly, the back side **30** includes a back flap **54** pivotally carried along back hinge **56**, with the other boundaries of the back flap **54** being defined by a U-shaped back flap margin **58**, which also may be a line of weakening, a line of separation, or a free margin. Preferably, the front side **28** extends over the frangible ampule **14** as illustrated in FIG. **2**, so that the channel **16** is beneath and extends beyond both the front hinges **46** and **48** and the back hinge **56**. Further, the back flap **54** is positioned over and thus preferably opens to reveal only the first slide section **20**, thereby inhibiting confusion which might be caused by application of the stool or other specimen to the control or second slide section **22**.

The center web **26** is cut or formed to present at least one and preferably two or more web openings **60** and **62** defining therewithin the first slide viewing section **64** for viewing the test results when the developer is applied to the first slide section **20** and the second slide viewing section **66** for viewing the results of the application of the developer to the control or second slide section **22**. The front flaps **42** and **44** and the back flap **54** are positioned in covering relationship to the first slide test section **20** and the second slide test section **22** so that a either front flap **42** or **44** may be pivoted along its respective hinge to expose either the first slide viewing section **64** or the second slide viewing section **66**, but only access to the back side of the first slide section is permitted for applying a tissue specimen such as a stool smear thereto. This arrangement permits both the first and second slide sections **20** and **22** to be selectively exposed for viewing of the test results once the ampule is crushed, but only the test slide, i.e. the first slide section **20** to be accessible from the back for application thereon of the tissue, stool or other sample, while the ampule **14** remains covered. Only the viewing sections **64** and **66** are exposed when either of the front flaps **42** and **44** are lifted, or a slightly larger area of the first test slide **20** when the back flap **54** is lifted. The panel **36** presents a depth for receiving the ampule **14** and channel **16** between the front side and the back side.

The ampule **14** includes a body **68** provided of a transparent frangible material such as glass or rigid synthetic resin material. As shown in the drawings, the body **68** of the ampule **14** is in the shape of an oblate or flattened hemisphere although other shapes could also be used. The ampule **14** also includes a developer **70** enclosed within the body **68**. The specific developer **70** used will depend on the type of test to be conducted, but by way of example, a stabilized aqueous solution of less than 5% hydrogen peroxide in 75% ethanol is useful in connection with testing for the presence of blood in stool samples. The volume of developer **70** contained within the ampule **14** is sufficient to flow through the channel and communicate with the first and second slide sections, with volumes of less than 1 ml typically being sufficient as only a drop or two of the developer must communicate with the slide **18** which acts through capillary action to deliver the developer thereacross. In the embodiment shown in FIG. **1**, two ampules **14**A and **14**B are provided, while in the embodiment shown in FIGS. **3** and **4**, a single ampule **14** communicates with separate slide sections **20** and **22** through the bifurcated channel.

The ampule **14** is contained within the channel **16** which is transparent and preferably of a resilient synthetic resin material resistant to breakage, such as high density polyethylene. The channel **16** is mounted by gluing or mechanical attachment to the center web **26** and includes a blister **72** for receiving the ampule **14** and at least one canal **74** in fluidic communication with the interior **76** of the blister **72** and leading to slide **18**. The blister **72** is substantially greater in volume than a canal **74** buy only relatively slightly greater in volume than the body **68**, such that the tubular canal **74** retains only a small fraction of any developer displaced when the blister **72** is compressed to crush and fracture of the ampule **14**. The canal **74** presents a proximate open end **78** communicating with the interior **76** of the blister but not into ampule **14**, and a remote open end **80** adjacent slide **18**. In the first embodiment shown in FIGS. **1** and **2**, each ampule **14**A and **14**B are resident within respective separate blisters **72**A and **72**B of segregated channels **16**A and **16**B, each channel having its own respective canal **74**A and **74**B for communicating the developer to separate first and second slide sections **20** and **22**. The blisters **72**A and **72**B may be operatively connected by a rigid press bar **82** overlying each blister in spanning relationship.

In the second embodiment of the test card **10**A shown in FIGS. **3** and **4**, the channel **16** has a single blister **72** communicating with two separate canals **74**C and **74**D each leading to separate first and second slide sections **20** and **22**. Further, each of the canals **74**C and **74**D may be provided with a check valve **84** positioned at the proximate open end **78**, as shown in FIG. **4**. The check valve **84** need not be sophisticated, but may merely be a restriction in the diameter of the canal **74** whereby the flow of developer under pressure from the channel to the slide is able to flow therepast, whereas after expression from the blister **72** and removal of pressure, the restriction serves as a check valve sufficient to inhibit backflow of unpressurized developer back into the blister or eventual passage from one canal to another.

The slide **18** is preferably presented in separate first and second slide sections **20** and **22**, and the carrier **12** may additionally carry separate control sections. In the case of test slides for measuring the presence of blood in the stool, the slide sections **20** and **22** may be of the same or separate sheets of thin permeable and absorbent tissue paper impregnated with a chromatographic reagent. One acceptable reagent for such uses is guaiac. When a control area is desired, the second slide section **22** may be provided as a positive control section impregnated with a substance which reacts with guaiac when the developer is applied, e.g., hemoglobin. A remote negative control section which is isolated from the test and positive control sections of the slide may be positioned remotely on the carrier and receive no guaiac-reactive substance thereon. Additional canals **74** may be provided to fluidically connect the blister to separate positive and negative control areas.

In order to use the frangible ampule specimen test card **10** of the present invention, the user need only lift the back flap **54** of the carrier **12** and smear a specimen, such as a stool specimen, onto the back side of the first slide section **20**. The user then lifts the front flaps **42** and **44** and presses on the blister **72** to fracture the ampule **14**, causing developer **70** to be placed under pressure and flow into the interior of the blister **72** and the remainder of the channel **16**. The applied pressure reduces the volume of the blister **72** sufficiently to cause the developer to flow into the canal or canals to the test section, e.g. first slide section **20** on the front side thereof opposite the smeared specimen. In the case of the first embodiment shown in FIGS. **1** and **2**, the application of pressure to the press bar **(82)** serves to collapse the blister and fracture the ampule in each channel **16**A and **16**B. In the second embodiment shown in FIGS. **3** and **4**, the pressure applied by the users thumb to the single blister is sufficient to direct the flow of developer past the check valves of the respective canals **74**C and **74**D and then to the first and second sections **20** and **22**. In both embodiments, the first slide section **20**, being preferably white and impregnated with guaiac, then turns blue when the developer **70** diffuses through the first slide **18** and moistens that portion of the slide **18** in contact with the stool specimen if blood is present, but does not change color and remains white if no hemoglobin is detected. As a control, when the developer **70** flows to the second slide section **22**, which is not only impregnated with guaiac but also with hemoglobin, the second slide section **22** should always turn blue if the developer **70** is functioning and the second slide section is properly impregnated. After the physician or other medical personnel view the test, the flaps **42**, **44** and **54** are closed and the test card **10** is disposed.

Although preferred forms of the invention have been described above, it is to be recognized that such disclosure is by way of illustration only, and should not be utilized in a limiting sense in interpreting the scope of the present invention. Obvious modifications to the exemplary embodiments, as hereinabove set forth, could be readily made by those skilled in the art without departing from the spirit of the present invention.

The inventor hereby states his intent to rely on the Doctrine of Equivalents to determine and assess the reasonably fair scope of his invention as pertains to any apparatus not materially departing from but outside the literal scope of the invention as set out in the following claims.

What is claimed is:

1. An occult blood specimen test card for testing medical specimens comprising:

a carrier of material for inhibiting the passage of liquid therethrough;

said carrier including a central web having an opening for defining therewithin a slide section, a front side and a back side, said front side having a front flap hingably mounted thereon in covering relationship to said slide section;

said slide section having a test slide and a control slide of fluid permeable paper impregnated with a reagent mounted on a carrier;

a first ampule presenting a first frangible body and containing therewithin a quantity of developing liquid;

a first channel mounted on the carrier and including a first flexible blister enclosing therewithin said first ampule and a first tubular canal presenting a proximate open end fluidically communicating with the first blister and a remote open end positioned proximate the test slide for conveying developing liquid from the first blister to the test slide upon compression of the first blister to fracture said first ampule and expel developing liquid from the first blister through the first canal; and

said first channel further including a second tubular canal presenting a proximate open end fluidically communicating with the first blister and a remote open end positioned proximate said control slide for conveying developing liquid from the first blister to said control slide upon compression of said first blister to fracture said first ampule and expel developing liquid from said first blister through said second canal.

2. A card as set forth in claim **1**, said back side having a back flap hingably mounted thereon in covering relationship to said slide section.

3. A card as set forth in claim **2**, wherein said slide section is positioned between said center web and said back side.

4. A card as set forth in claim **1**, wherein said control slide is provided with both said reagent and a control substance for providing a chromatographic indication in the presence of the developing liquid.

5. A card as set forth in claim **1**, said slide section being impregnated with guaiac and said developing solution including a solution of hydrogen peroxide.

6. A card as set forth in claim **5**, wherein said carrier presents openings defining a test slide and a control slide discrete from said test slide, said first channel including a second canal having a proximate open end in fluidic communication with said first blister and a remote open end positioned in spaced relationship from said remote open end of said first canal whereby the remote open end of said first canal is positioned proximate said test slide and the remote open end of said second canal is positioned proximate said control slide.

7. A card as set forth in claim **6**, including a restriction defining a check valve positioned at said proximate open end of each of said first and second canals.

8. A card as set forth in claim **1**, wherein said first channel is unitary and formed of a resilient synthetic resin material.

9. A card as set forth in claim **1**, wherein said frangible ampule is of glass.

10. A card as set forth in claim **1**, wherein said carrier presents openings defining a test slide and a control slide discrete from said test slide, the remote open end of the first canal being positioned proximate said test slide and the remote open end of said second canal being positioned proximate said control slide.

11. A card as set forth in claim **10**, including a rigid press bar positioned exteriorly of and in spanning relationship over said first and second blisters.

12. An occult blood specimen test card for detecting the presence of hemoglobin comprising:

a carrier presenting a front side, a back side and a center web positioned between said front side and said back side, said center web presenting at least one opening therein, said front side and said back side each having hingably mounted flaps thereon positioned in covering relationship to said opening;

a test slide of permeable paper impregnated with a chromatographic reagent, said test slide being positioned between said front side and said back side of said carrier and within said opening to present a first test section;

a control slide of permeable paper impregnated with a chromatographic reagent, said control slide being positioned between said front side and said back side of said carrier and within said opening to present a second test section;

a first ampule having a frangible body containing therein a quantity of a developing solution;

a first channel mounted on said carrier and including a first flexible blister defining an interior and enclosing therein said first ampule, a first canal presenting a proximate open end fluidically communicating with said interior and a remote open end positioned proximate said first test section for directing developing solution to said first test section when said first ampule is crushed within said interior to thereby cause a chromatographic reaction by said reagent in response to the presence of hemoglobin on said test slide; and

a second canal presenting a proximate open end fluidically communicating with said interior and a remote open end positioned proximate said second test section for directing developing solution to said second test section when said first ampule is crushed within said interior to thereby cause a chromatoaraphic reaction by said reagent in response to the presence of hemoglobin on said control slide.

13. An occult blood specimen test card as set forth in claim **12**, and including a second opening defining a second test section, a second ampule having a frangible body containing therein a quantity of developing solution, and a second channel mounted on said carrier and including a second flexible blister defining an interior and enclosing therein said second ampule and a second canal presenting a proximate open end fluidically communicating with said interior and a remote open end positioned proximate said second test section for directing developing solution to said second test section when said second ampule is crushed within said interior to thereby cause a chromatographic reaction by said reagent in response to the presence of hemoglobin on said control slide.

14. An occult blood specimen test card as set forth in claim **12**, and including a second opening defining a second test section, said first channel including a second canal having a proximate open end communicating with the interior of said first blister and a remote open end proximate said second test section for directing developing solution to said second test section to thereby cause a chromatographic reaction by said reagent in response to the presence of hemoglobin on said control slide.

15. A method of testing a stool sample to determine the presence of blood therein comprising:

providing an integrated test card including a carrier, a test slide of permeable material impregnated with a chromatographic reagent and carried by the carrier, a control slide of permeable material impregnated with a chromatographic reagent and carried by the carrier, at least one ampule having a frangible body containing therein a liquid developer, a channel mounted on said carrier and including a flexible blister defining an interior in which said ampule is located, a first canal fluidically communicating said interior with said test slide, and a second canal fluidically communicating said interior with said control slide;

applying a stool sample on said test slide; and

compressing said blister to crush said ampule and direct a flow of developer through said first canal to said test slide and through said second canal to said control slide for causing a chromatographic reaction on said test slide in the presence of blood on said test slide.

16. The method of claim **15**, wherein said control slide is provided with both said reagent and a control substance for providing a chromatographic indication in the presence of the developing liquid.

17. The method of claim **16**, further comprising the step of comparing said control slide to said test slide for determination of the presence of blood in said test slide.

18. An occult blood specimen test card for testing medical specimens comprising:

a carrier of material for inhibiting the passage of liquid therethrough;

said carrier including a central web having an opening for defining therewithin a slide section, a front side and a back side, said front side having a front flap hingably mounted thereon in covering relationship to said slide section;

said slide section having a test slide and a control slide of fluid permeable paper impregnated with a reagent mounted on a carrier;

a first ampule presenting a first frangible body and containing therewithin a quantity of developing liquid;

a second ampule presenting a second frangible body and containing therewithin a quantity of developing liquid;

a first channel mounted on the carrier and including a first flexible blister enclosing therewithin said first ampule and a first tubular canal presenting a proximate open end fluidically communicating with said first blister and a remote open end positioned proximate the test slide for conveying developing liquid from said first blister to said test slide upon compression of said first blister to fracture said first ampule and expel developing liquid from said first blister through said first canal; and

a second channel mounted on said carrier discretely from said first channel and having a second flexible blister and including a second tubular canal mounted on said carrier discretely from said first channel, said second channel containing therein said second ampule having a frangible body containing a quantity of said developing liquid, said second canal fluidically communicating said second blister to said control slide.

19. A card as set forth in claim **18**, said back side having a back flap hingably mounted thereon in covering relationship to said slide section.

20. A card as set forth in claim **19**, wherein said slide section is positioned between said center web and said back side.

21. A card as set forth in claim **18**, wherein said control slide is provided with both said reagent and a control substance for providing a chromatographic indication in the presence of the developing liquid.

22. A card as set forth in claim **18**, said slide section being impregnated with guaiac and said developing liquid including a solution of hydrogen peroxide.

23. A card as set forth in claim **22**, wherein said carrier presents openings defining a test slide and a control slide discrete from said test slide, said first channel including a second canal having a proximate open end in fluid communication with said first blister and a remote open end positioned in spaced relationship from said remote open end of said first canal whereby the remote open end of said first canal is positioned proximate said test slide and the remote open end of said second canal is positioned proximate said control slide.

24. A card as set forth in claim **18**, wherein said first channel is unitary and formed of a resilient synthetic resin material.

25. A card as set forth in claim **18**, wherein said frangible ampule is made of glass.

26. A card as set forth in claim **18**, wherein said carrier presents openings defining a test slide and a control slide discrete from said test slide, the remote open end of said first canal being positioned proximate said test slide and the remote open end of said second canal being positioned proximate said control slide.

27. An occult blood specimen test card for testing medical specimens comprising:

a carrier of material for inhibiting the passage of liquid therethrough;

said carrier including a central web having an opening for defining therewithin a slide section, a front side and a back side, said front side having a front flap hingably mounted thereon in covering relationship to said slide section;

said slide section having a test slide and a control slide of fluid permeable paper impregnated with a reagent mounted on a carrier;

a first ampule presenting a first frangible body and containing therewithin a quantity of developing liquid;

a second ampule presenting a second frangible body and containing therewithin a quantity of developing liquid;

a first channel mounted on the carrier and including a first flexible blister enclosing therewithin said first ampule and a first tubular canal presenting a proximate open end fluidically communicating with said first blister and a remote open end positioned proximate the test slide for conveying developing liquid from said first blister to said test slide upon compression of said first blister to fracture said first ampule and expel developing liquid from said first blister through said first canal; and

a second channel mounted on said carrier discretely from said first channel and including a second flexible blister enclosing therewithin said second ampule and a second tubular canal presenting a proximate open end fluidically communicating with said second blister and a remote open end positioned proximate said control slide for conveying developing liquid from said second blister to said control slide upon compression of said second blister to fracture said second ampule and expel developing liquid from said second blister through said second canal.

28. A card as set forth in claim **27**, said back side having a back flap hingably mounted thereon in covering relationship to said slide section.

29. A card as set forth in claim **28**, wherein said slide section is positioned between said center web and said back side.

30. A card as set forth in claim **28**, wherein said carrier presents openings defining a test slide and a control slide discrete from said test slide, said first channel including a second canal having a proximate open end in fluidic communication with said first blister and a remote open end positioned in spaced relationship from said remote open end of said first canal whereby the remote open end of said first canal is positioned proximate said test slide and the remote open end of said second canal is positioned proximate said control slide.

31. A card as set forth in claim **27**, wherein said control slide is provided with both said reagent and a control substance for providing a chromatographic indication in the presence of the developing liquid.

32. A card as set forth in claim **27**, said slide section being impregnated with guaiac and said developing liquid including a solution of hydrogen peroxide.

33. A card as set forth in claim **27**, wherein said first channel is unitary and formed of a resilient synthetic resin material.

34. A card as set forth in claim **27**, wherein said frangible ampule is made of glass.

35. A card as set forth in claim **27**, wherein said carrier presents openings defining a test slide and a control slide discrete from said test slide, the remote open end of said first canal being positioned proximate said test slide and the remote open end of said second canal being positioned proximate said control slide.

* * * * *

Exhibit F

United States Patent [19]

Slover et al.

[11] **4,445,235**

[45] **May 1, 1984**

[54] **STOOL SPECIMEN COLLECTOR**

[76] Inventors: **Pearl Slover**, 345 Sybil Ave., San Leandro, Calif. 94577; **Robert R. Moore**, 1897 National Ave., Hayward, Calif. 94545

[21] Appl. No.: **417,197**

[22] Filed: **Sep. 13, 1982**

[51] **Int. Cl.**[3] **A47K 11/00**

[52] **U.S. Cl.** **4/144.2**; 4/144.1; 4/451; 4/455

[58] **Field of Search** 4/661, 144.1, 144.2, 4/301, 450, 451, 452, 455

[56] **References Cited**

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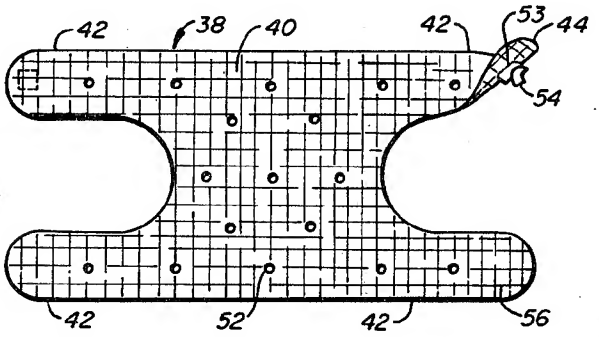
Primary Examiner—Henry K. Artis

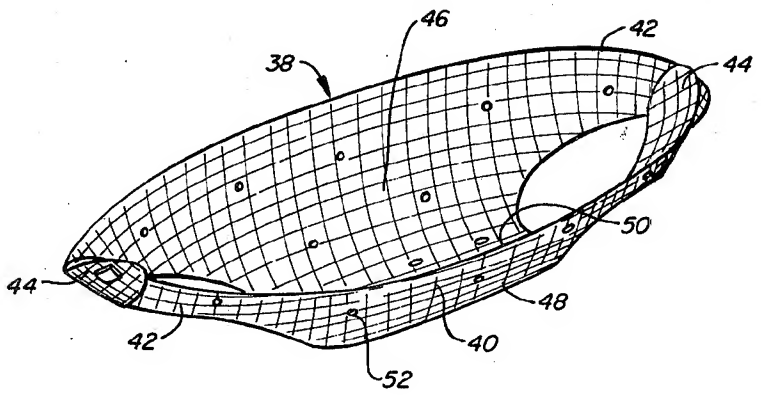
Attorney, Agent, or Firm—Bielen & Peterson

[57] **ABSTRACT**

A stool specimen collector for collecting a medical patient's feces for laboratory examination and test, the collector having a subtantially impervious receptacle with a pair of side straps having an adhesive surface portion for contact adhesion to the top surface of a conventional toilet seat, the container being suspended below the toilet seat and above the surface of the toilet water, positioned to catch and retain a fecal specimen.

3 Claims, 4 Drawing Figures





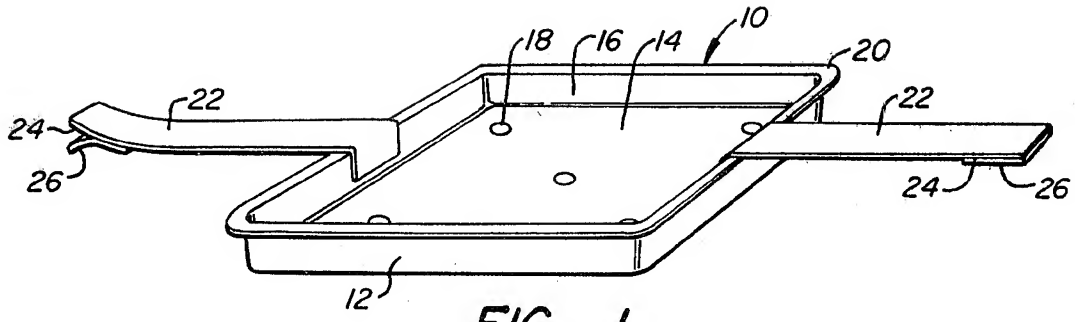


FIG. 1.

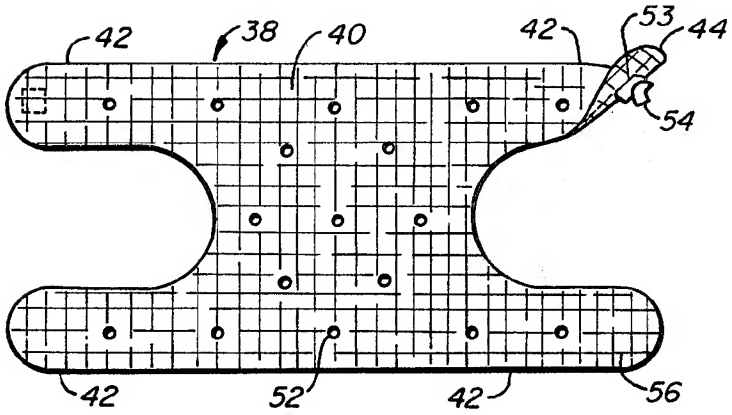


FIG. 3.

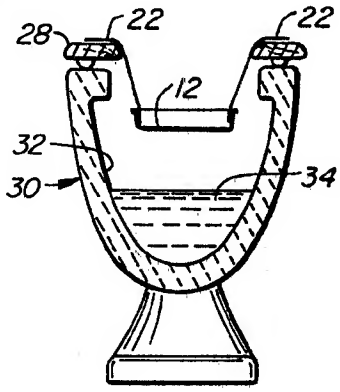


FIG. 2.

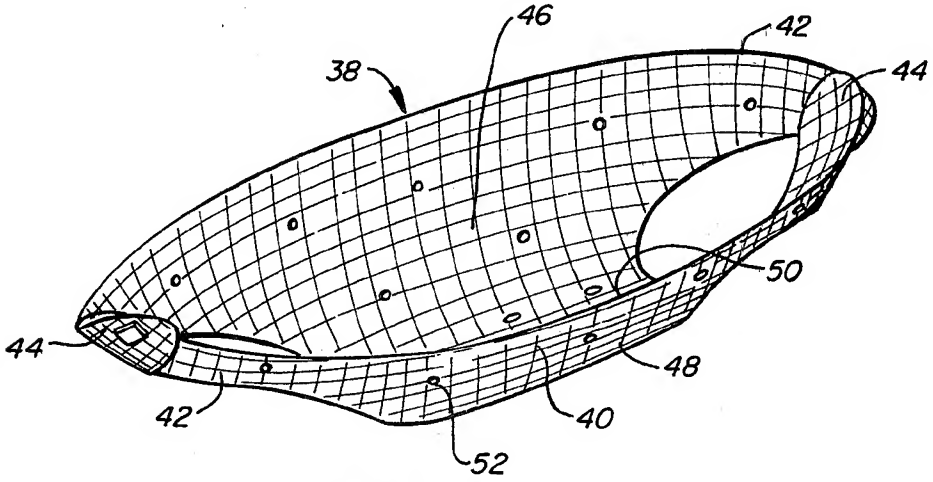


FIG. 4.

STOOL SPECIMEN COLLECTOR

BACKGROUND OF THE INVENTION

This invention relates to a collector device for collection of a medical patient's stool specimen for examination and testing by a physician or a medical test laboratory. For many illnesses, particularly stomach and intestinal tract disorders, it is a conventional and common practice to examine and test a patient's feces for diagnostic and treatment purposes. Also, some post-operative monitoring procedures include periodic examination of the patient's feces.

Stool specimen collection has in the past been accomplished by the patient, with or without assistance, holding a pan or other container under the rectum, either in a squatting position or while sitting on a conventional toilet. The awkward nature of such procedures is evident. Specimens have also been collected by retrieving the specimen from the toilet water with a scoop after a patient has defecated. This method has the potential of contaminating the specimen with unclean toilet water, which may in some occasions adversely affect a test or analysis.

The past methods of stool collection are not only awkward to perform, but are somewhat embarrassing to the patient. Further, such methods commonly use a container that is not self draining, causing added problems of spillage, and subsequent separation for analysis, or that is not disposable, causing problems of possible contamination.

The stool specimen collection device of this invention is constructed for convenient attachment to a conventional toilet seat with a receptacle centrally located below the seat, above the water level in the toilet. The collection device is fabricated from a material that is water resistant, or at least not structurally denegrated by water or fluid discharge by the patient. The device is preferably constructed with a receptacle that includes perforations to allow drainage of liquids that may otherwise be inadvertently entrapped in the receptacle. The drainage feature facilitates removal of the collector from attachment to the seat, and placement of the collector and contents into a sealable container for transport to the examination facility.

The collector device of this invention is designed primarily for the convenience of the users, allowing the patient to defecate in a normal comfortable manner using a conventional flush toilet, and allowing the patient or medical staff to remove and package the specimen quickly, conveniently and without contaminating auxiliary components such as pans, trays or the like. The relief of the patient's tension and anxiety alone allows the stool sample procedure to be accomplished with minimum delay and complication. The simplicity of the procedure allows for office out-patient or home use of the device for collection of the necessary specimens.

SUMMARY OF THE INVENTION

The stool specimen collector of this invention comprises a receptacle and an integral attachment means for suspending the receptacle below the seat of a conventional toilet and above the level of the toilet water. The receptacle is designed to catch and retain a patient's feces during the normal process of defecating. The collector is fabricated from materials that are water resistant or that have a wet strength, and that can be easily disposed. Preferably, the material is biodegradable or combustible without toxic fumes.

The design of the receptacle is preferably such that inadvertent passage of urine onto the collector will be drained through the receptacle for convenient handling of the collector during removal and transport for examination. This feature is most effectively accomplished by the inclusion of a plurality of small drainage holes in the receptacle of the collector. To maximize the retention of the patient's stool specimen in the receptacle particularly where the collected specimen may be of a loose or wet consistency and would pass through a net-type receptacle design, the holes are relatively small and uniformly spaced over the surface area of the receptacle.

The attachment means of the collector is constructed of strap members fixed to, or integral with, the receptacle. The strap members preferably include adhesive segments to directly adhere the strap members to the surface of the toilet seat or to the surface of the top rim of the toilet bowl. While the strap members could include or comprise thin laces to tie the suspended receptacle to the seat, the method of adhesive attachment is preferred for the convenience of attachment of the collector to the toilet apparatus and removal therefrom.

These and other features of the stool specimen collector are described in greater detail in the description of the preferred embodiments hereafter.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a perspective view of one embodiment of the stool specimen collector.

FIG. **2** is a side elevational view of the collector of FIG. **1** placed in position on a conventional toilet, illustrated in partial cross-section.

FIG. **3** is a top plan view of an alternate embodiment of a specimen collector, before assembled into a preferred configuration for placement in use.

FIG. **4** is a top plan view of the alternate embodiment of the collector placed in position for use on a conventional toilet.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring to FIG. **1**, a first embodiment of the stool specimen collector is shown and designated generally by the reference numeral **10**. The specimen collector is constructed with a shallow open receptacle **12** having a bottom **14** and peripheral sides **16** forming a tray-like structure. The receptacle **12** has a plurality of drain holes **18** through which fluid, received in the collector, can conveniently pass.

The receptacle **12** is preferably fabricated from a rigid or semi-rigid material that is water resistant or maintains its structural integrity when wet.

The wet strength requirement is necessary to prevent the weight of a wet stool specimen from splitting an untreated receptacle made, for example, of paper.

The receptacle may also become inadvertently wet from contact with the toilet water or upon passage of urine by the patient and must retain its strength when receiving the specimen.

The tray-like structure of the receptacle **12** shown in FIGS. **1** and **2** is constructed from a P.V.A. plastic that is eventually dissolvable in water but which will maintain a structural integrity, even when wet, for the expected period of use as a specimen collector. Alter-

nately, the receptacle can be constructed from a cardboard material treated for water resistance, which also permits biodegradability after expected duration of use.

While other plastic materials may be employed, it is desired that they at least be combustible or heat degradable without toxic emission as disposal of the semi-rigid collector may likely be accomplished by burning in a hospital furnace.

The sides **16**, bottom **14** and a top rim **20** of the receptacle **12** are integrally fabricated in a stamping process. The drain holes **18** are punched in the bottom **14** of the receptacle, and are sufficiently large for drainage of inadvertently passed urine, without loss of the fecal specimen through the holes.

Connected by an adhesive to two opposed sides of the collector are elongated straps **22**. The straps **22** are fabricated from a flexible treated paper and have a non-curing adhesive section **24** on the underside of the distal ends of the straps which, prior to use, are protected by peel-off backing strips **26**.

As illustrated in FIG. **2**, when the backing strip is removed, the adhesive ends of the straps **22** can be pressed against the top of a seat **28** of a conventional toilet **30**. The length of the straps are such that the receptacle **12** is centered within the bowl **32** of the toilet **30** below the seat and above the level of the toilet water **34**.

The patient simply defecates in a normal manner, preferably using restraint on discharge of urine. The feces is caught by the receptacle before falling to the water. There should be no added discomfort from sitting on the strap ends that adhere to the seat, particularly with an additional protective paper seat cover, as is common in most lavatories. The straps may, if desired, be adhered directly to the rim **36** of the toilet without affecting the positioning of the receptacle by proper adjustment of the straps **22**.

The collector is sized to be adequate for central positioning within the bowl with a maximum likelihood of catching the patient's discharge, yet allowing by-pass of a urinal discharge and toilet disposal of toilet paper. Conveniently, the size of the receptacle is one quarter to one half the diameter of the bowl at the level where the receptacle is located.

Where expense and convenience of disposability are important factors, a one piece paper collector is preferred. Referring to FIG. **3**, a flat pattern for a paper stool specimen collector, designated generally by the reference numeral **38**, is shown. The paper collector **38** has a central portion **40** with four projecting integral strap elements **42**, with two parallel elements projecting as a pair from each opposite side of the central portion.

As shown in FIG. **4**, the pairs of strap elements have distal ends **44** that are overlapped and secured together by an adhesive. The resultant structure, when supported by the opposed, mutually overlapped ends **44**, deforms the central portion into an open cradle receptacle **46** having a depressed center **48** and a raised periphery **50**.

To provide adequate drainage of water and other liquids, the flat pattern has a plurality of perforations or holes **52** punched through the paper material before interconnection of the distal ends. Although the distal ends may be connected by the user, it is preferred that they be connected during the manufacturing process to avoid confusion on arranging the strap elements on the toilet seat.

The two pairs of interconnected strap elements each have an adhesive pad **53** with a peel-off strip **54** adhered to one side of the overlapped ends. In a similar manner to the previously described embodiment, the backing strip **54** is removed and the strap pairs adhered to the top surface of the seat of a conventional toilet.

The paper collector is fabricated from a flexible water-resistant paper with an internal fibre reinforcement web **56**. The material, composition and size of the collector pattern allows the entire collector and any remaining stool specimen to be disposed by flushing down the toilet. The water resistance of the material is such that it eventually deteriorates, allowing the collector to freely pass through conventional waste water systems. The collector and specimen may be placed in a plastic or water resistant paper bag (not shown) for temporary storage or transport, after the specimen has been collected. The described features make the paper collector compact for storage in a folded condition, easy to use by the average patient, and convenient to dispose.

While on the foregoing embodiments of the present invention have been set forth in considerable detail for the purposes of making a complete disclosure of the invention, it may be apparent to those of skill in the art that numerous changes may be made in such detail without departing from the spirit and principles of the invention.

What is claimed is:

1. A stool specimen collector for use by a medical patient for collection of a stool specimen comprising:

a flat, flexible water-resistant material having a perforated central portion with four projecting, integral strap elements with two shaped parallel elements projecting in a pair from each opposite side of said central portion, said strap elements having distal ends with attachment means for attaching the strap elements to a toilet, wherein on use of said collector, said distal ends in each pair are connected and attached to opposite sides of the top of a conventional toilet, said central portion forming an open cradle receptacle with a depressed center and raised periphery, said receptacle being supported below the seat of the toilet, above the bowl water for receiving the feces of a user and wherein said center portion and said four projecting integral strap elements are formed from a one piece pattern.

2. The stool specimen collector of claim **1** wherein said one piece pattern is fabricated from a paper material.

3. The stool specimen collector of claim **2** wherein said distal ends in each pair are preconnected and include adhesion means for adhering the preconnected ends to the toilet.

* * * * *

Exhibit G

US005215713A

United States Patent [19]

Steinbiss

[11] Patent Number: 5,215,713

[45] Date of Patent: Jun. 1, 1993

[54] **TEST KIT FOR DETERMINING AN ANALYTE IN A PASTY SAMPLE**

[75] Inventor: **Joachim Steinbiss**, Lorsch, Fed. Rep. of Germany

[73] Assignee: **Boehringer Mannheim GmbH**, Mannheim; Fed. Rep. of Germany

[21] Appl. No.: **727,487**

[22] Filed: **Jul. 9, 1991**

[30] **Foreign Application Priority Data**

Jul. 17, 1990 [DE] Fed. Rep. of Germany 4022655

[51] **Int. Cl.**[5] **G01N 21/01**; G01N 33/00

[52] **U.S. Cl.** **422/61**; 422/56; 422/57; 422/58; 435/805; 435/810; 436/66; 436/165; 436/169

[58] **Field of Search** 422/61, 58, 100, 101, 422/104, 56, 55, 57; 435/810, 805; 436/66, 169, 165, 805, 808

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Primary Examiner—James C. Housel
Assistant Examiner—Long V. Le
Attorney, Agent, or Firm—Felfe & Lynch

[57] **ABSTRACT**

Test kit **10** for determining an analyte in a pasty sample, in particular in stool. It contains a capillary-active fluid transport section **11** which leads from an eluant application zone **12** via a sample application zone **14** to an eluate reception zone **15**, together with analysis section containing reagents which react with the analyte and include a component producing a test signal. Improved reliability of the analysis with simplicity of manufacture and ease of handling is achieved by the fact that the fluid transport path **11** between the eluant feed zone **12** and the sample field **23** is formed as a delay section **13**. The sample application zone **14** is provided with a sample layer **16a** with a sample field **23** for the application of the sample.

11 Claims, 3 Drawing Sheets

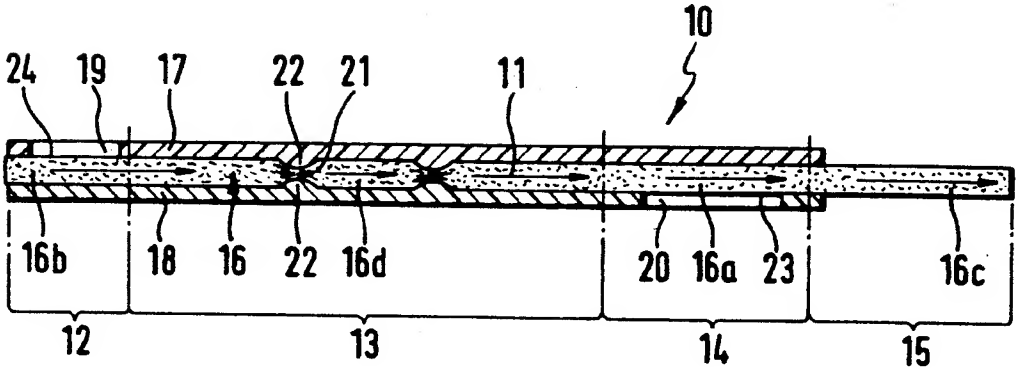


Fig. 1

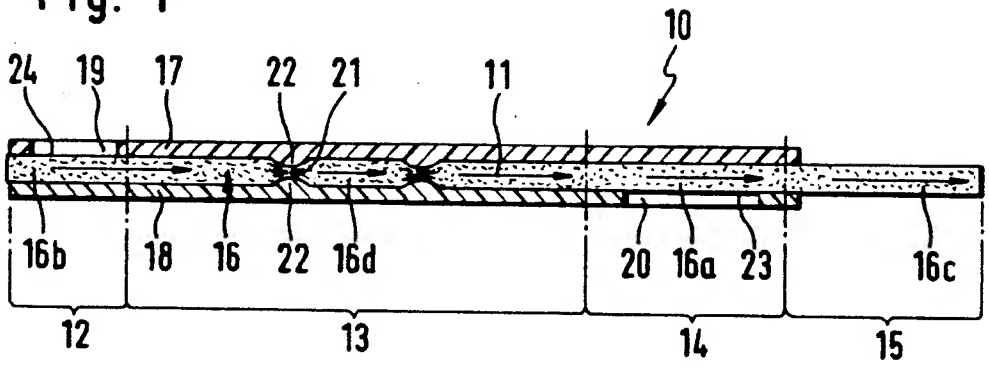


Fig. 2

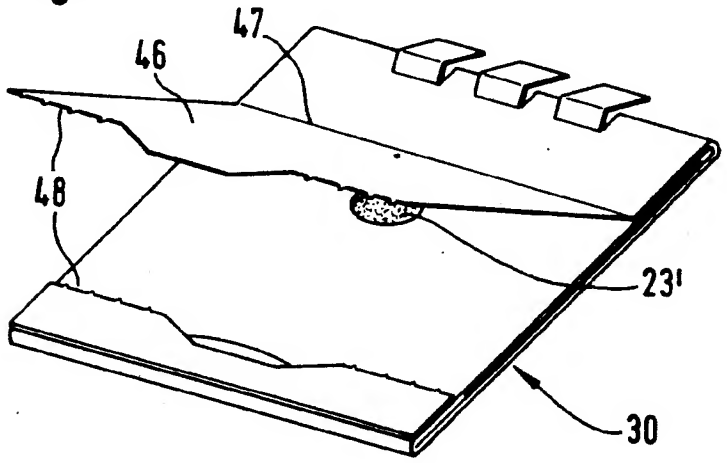
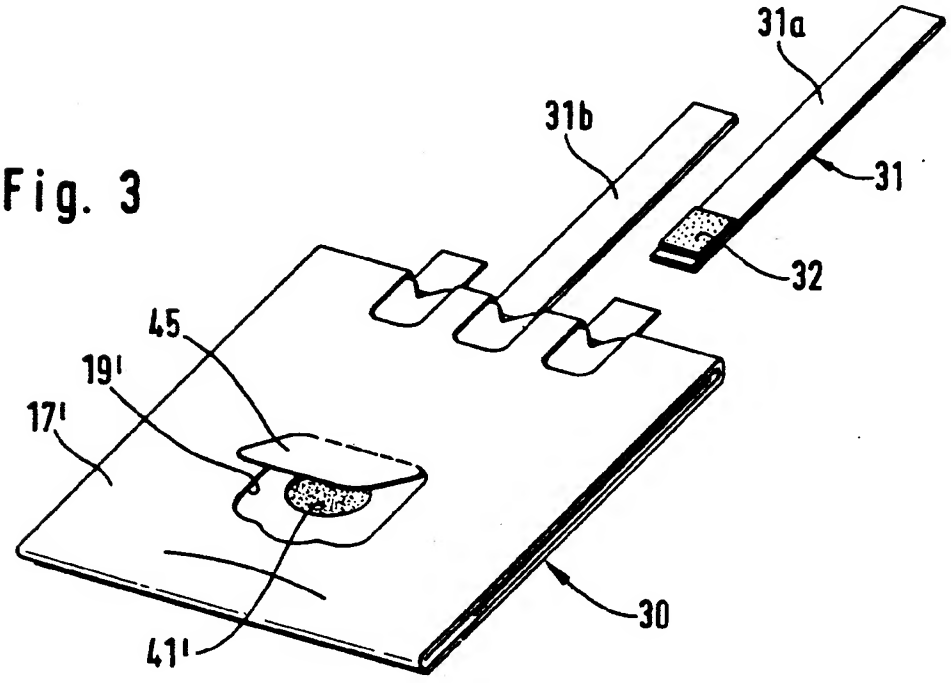


Fig. 3



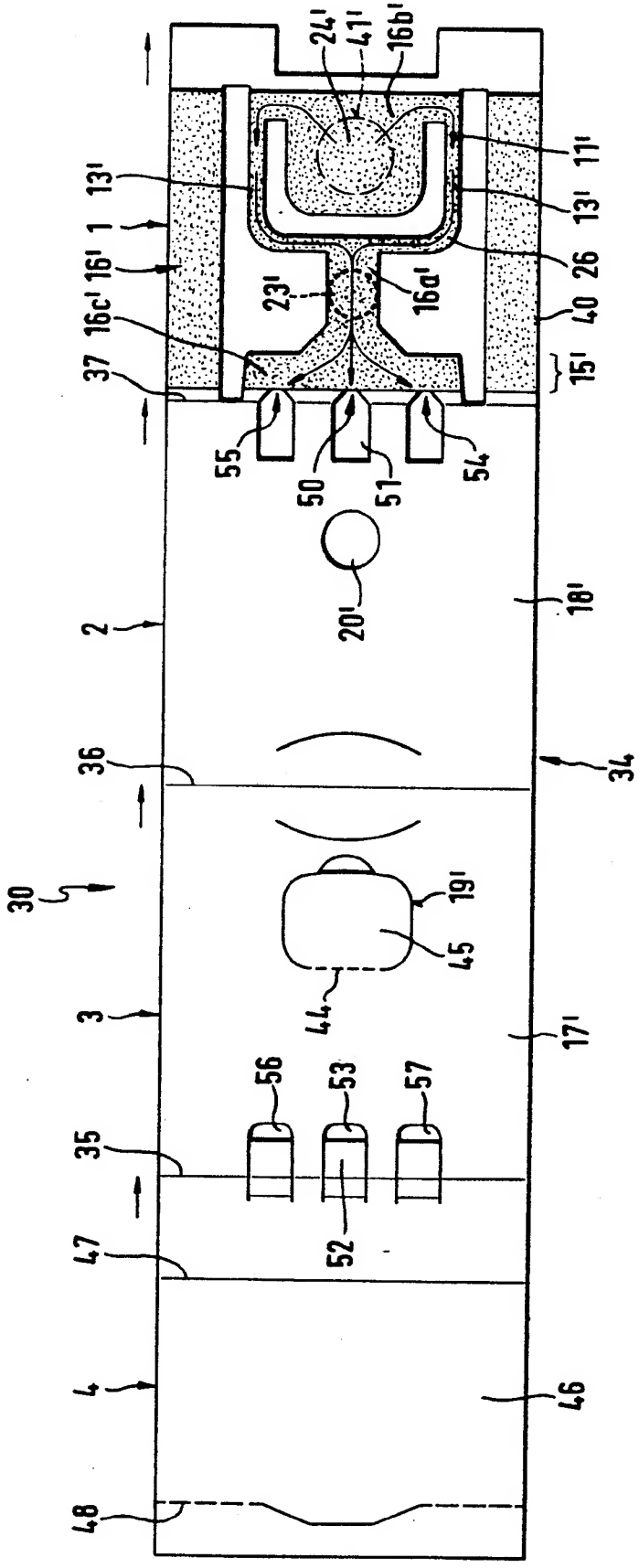

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Fig. 4

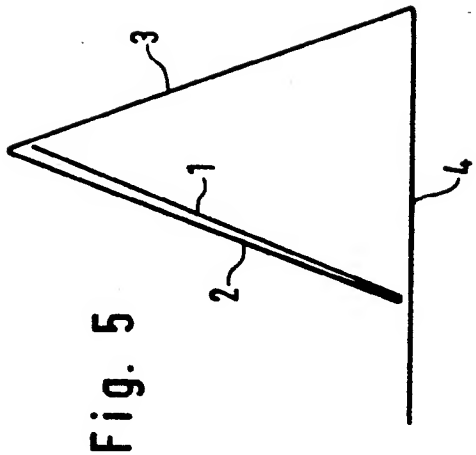

Fig. 5

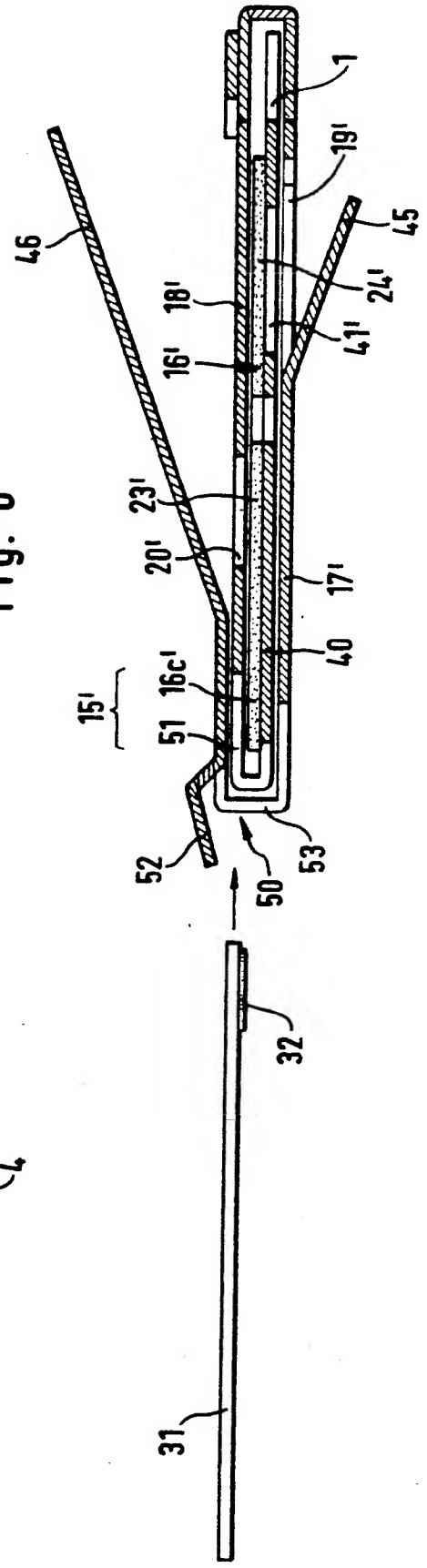

Fig. 6

TEST KIT FOR DETERMINING AN ANALYTE IN A PASTY SAMPLE

The invention relates to a test kit for determining an analyte in a pasty sample, in particular in stool, comprising a fluid transport section consisting of capillary-active porous material, which leads from an eluant application zone via a sample application zone to an eluate reception zone, and analysis means containing reagents which react with the analyte and include a component producing a test signal. The sample application zone is provided with a sample layer with a sample field for the application of the sample.

Such a test kit is known from published European patent application EP-A-0 291 843. The test kit described there serves, just like the present invention, mainly for the analysis of stool, but can also be used with advantage for other sample materials, in particular homogenates of animal and human tissue samples or galenic suspensions for purposes of pharmaceutical analysis. In general the invention relates to the analysis of pasty, spreadable samples which contain solid constituents, in particular for medical purposes. Discussion will be confined for the sake of simplicity, but without restricting the general import, to the analysis of stool.

The term "test kit" denotes combinations of reagents and adjuvants required for an analysis. Although a test kit consists in most cases of several units, one-piece analysis elements are also available for stool tests, which must likewise be regarded as test kits in the context of the present invention.

The test kit described in the above-mentioned European patent application is suitable in particular for immunological analyses of stool constituents (in particular of HSA, human serum albumin). Compared with the immunological stool tests known to date, it is distinguished above all by ease of handling. The stool sample is placed on the sample field, where it penetrates partially into the porous sample layer. After this an elution fluid (for example, aqueous buffer solution) is applied in the eluant application zone to the fluid transport path, which consists preferably of a single layer material. It chromotographs along the fluid section, eluting soluble constituents out of the sample. The eluate continues to chromatograph in the eluate reception zone on the basis of the capillary forces acting in the fluid transport path. Analysis means are provided there, which react with the analyte and produce a test signal which preferably consists of a colour change. Other test signals (e.g. fluorescence) are also possible, however, depending on the analysis means used.

The object of the present invention is to provide a test kit for the analysis of pasty samples which has improved analytical properties.

The object is achieved with a test kit of the kind described in the preamble by the fact that the fluid transport path is provided with a delay section between the eluant feed zone and the sample field.

Due to the delay section the fluid transport rate in the sample layer is reduced in a controlled manner. If the sample layer is regarded as a separate fluid transport element, the flow rate is determined primarily by the fluid transport properties of its material. On the one hand these are dependent on the capillary forces in the layer, which are determined by the surface properties of the layer material and the size of the capillaries therein, and on the other hand they are determined by the flow resistance in the layer. In practice, however, these parameters cannot be specified and optimized independently of one another. In particular, the layer material also has to be selected according to other critical values such as, for example, strength, relatively small layer thickness (preferably less than 0.5 mm, particularly preferably less than 0.3 mm) and sufficient porosity for the penetration of the sample.

In the case of the previously known test kit the sample field (or in a preferred embodiment the first of a plurality of sample fields) follows immediately downstream the eluant application zone. The flow rate at which the eluant flows through the applied sample in the sample layer is consequently for practical purposes determined only by the latter's own properties. In the case of the present invention the fluid transport rate of the delay section is substantially lower. Since the whole fluid transport path of the test kit forms in close approximation a closed flow path, the flow rate (expressed as mass transfer, for example in µl/sec) is the same throughout the fluid transport path. The delay section therefrom enables the fluid transport rate within the sample layer in the region of the sample field to be fixed at an optimal value for the particular test independently of the sample layer's own fluid transport properties.

A delay section in the context of the present invention has the property that, considered in isolation, it possesses a lower fluid transport rate for the eluant than the sample layer in the region of the sample field (again considered in isolation). The flow rate in the delay section should preferably be smaller by a factor of at least 2, preferably by a factor of at least 5, than the flow rate in the region of a sample field of the sample layer tested in isolation.

The reduced fluid transport rate in the fluid transport section can be achieved in various ways. In the simplest case the delay section can consist simply of a prolonged section between eluant application zone and sample field (or in the case of several sample fields the first sample field). It is preferably longer than the longitudinal dimension of the sample field in the flow direction, particularly preferably at least twice as long.

The delay section can also consist of a capillary-active layer material which is different from the sample layer and transports the fluid more slowly. This is expensive in manufacturing terms, however, since in this case several layer materials have to be positioned along the fluid transport path and have to be so connected to one another that the fluid can pass over from the one layer into the other ("fluid contact").

Particularly preferred, therefore, is the use of a uniform layer material for delay section and sample layer, with a narrowing being provided in the region of the delay section. The narrowing can consist both of one or more constrictions perpendicular to the superficial extent of the layer material and of one or more sections of the layer material of reduced width.

The various realizations of the delay section can also be combined with one another.

An improved quality of analysis is achieved by means of the invention. The reduced flow rate of the eluant in the sample layer leads to an increase in the analyte concentration. In the context of the present invention it has been established that this is highly advantageous for the analytical quality, although on the one hand highly sensitive reagent systems are available for detecting with extraordinarily high sensitivity the analytes involved, and although on the other the delay section

increases the time required for the analysis and necessitates a complicated configuration of the fluid transport path. Due to the fact that, according to the invention, the elution properties are improved and at the same time a relatively insensitive test system is used, the number of false positive findings is considerably reduced and the required detection reliability nonetheless ensured, i.e. false negative findings are largely eliminated.

In addition the invention leads to an improved separation of the colored constituents of the stool which interfere with the analysis.

A particular advantage of the invention consists in the fact that the analysis result is largely independent of the degree of dryness of the sample. Whereas in the case of the previously known analysis elements with a relatively fresh sample the probability of false positive findings rose, or (with reduced detection sensitivity of the analysis system) the probability of false negative findings grew in the case of well dried-out samples, almost the same results are obtained according to the invention with virtually fresh samples and those stored for protracted periods.

Relatively insensitive, non-immunological, in particular enzymatic analyses can also be carried out with great reliability according to the invention.

It is found, surprisingly, that the reduction of the flow rate, although it leads to an enrichment of the analyte, does not lead in practice to a troublesome increase in concentration of the stool constituents interfering with the analysis.

In accordance with the invention, a test kit for determining an analyte in a pasty sample comprises a capillary-active fluid transport path which leads from an eluate application zone via a sample application zone to an eluate reception zone, in which the sample application zone has a sample layer having a sample field for the application of the sample. The test kit also includes analysis means containing reagents which react with the analyte and comprise a component producing a test signal. A section of the fluid transport path between the eluant feed zone and the sample field is formed as a delay section.

For a better understanding of the invention, together with other and further objects thereof, reference is made to the following description, taken in connection with the accompanying drawings, and its scope will be pointed out in the appended claims.

Referring now to the drawings:

FIG. **1** shows an overall view of a test kit according to the invention in cross-section;

FIG. **2** shows the sample collecting unit of a preferred embodiment viewed in perspective from the patient's side,

FIG. **3** shows a sample collecting unit as per FIG. **2** viewed in perspective from the doctor's side, including test carriers forming part of the test kit,

FIG. **4** is a view of a cardboard blank for manufacturing a sample collecting unit according to FIGS. **2** and **3**,

FIG. **5** is a folding scheme for FIG. **4**,

FIG. **6** is a longitudinal section through a sample collecting unit according to FIGS. **2** to **5**.

The laboratory model of a test kit **10** shown in FIG. **1** possesses a fluid transport path symbolized by the arrow **11**, which leads from an eluant feed zone **12** via a delay section **13** and a sample application zone **14** to an eluate reception zone **15**.

The fluid transport path **11** is in the preferred case shown formed from a single continuous insert part **16** consisting of a porous, capillary layer material, which is encased like a sandwich between two cover parts **17** and **18**. This embodiment is particularly easy to manufacture. In the context of the invention, however, different layer materials can also be used in the zones **12** to **15**, which are in fluid contact with one another. The fluid contact can moreover be produced not only by capillary-active porous layer materials, but also by capillary columns or tubes. The individual sections of the layer material in the zones **12** to **15** are labelled suction layer **16*b***, delay layer **16*d***, sample layer **16*a*** and eluate reception layer **16*c***.

The first cover part **17** and the second cover part **18** possess in each case a recess **19** or **20**, through which the fluid transport section is accessible in the eluant application zone **12** (suction layer **16*b***) or in the sample application zone **14** (sample layer **16*a***). The cover parts **17**, **18** are made of a moisture-proof layer material, for example coated cardboard or a stable plastics film. They can optionally also be manufactured as shaped parts (for example as shown in EP-A-0 291 843).

The delay layer **16*d*** consists in the preferred case shown in FIG. **1** of the same capillary-active flat layer material (of the insert part **16**) as the rest of the transport path. Its fluid transport cross-section is narrowed by constrictions **21** at which the layer material is compressed perpendicular to its surface dimension. In addition the delaying effect of the delay section **13** is based on the fact that it is relatively long. Its length preferably is at least 25% of the overall fluid transport path from the eluant application zone **12** to the eluate reception zone **15**.

In the case of the embodiment shown the constrictions **21** are fixed by corresponding raised profilings **22** on the cover parts **17**, **18**. This is only necessary, however, if the material of which the delay layer consists is so flexible that the constrictions **21** do not remain in position without a suitable fixing means.

For the carrying out of an analysis the test kit shown in FIG. **1** is held with the second cover part **18** at the top (i.e. the opposite position to that shown). The sample is applied to the sample field framed by the recess **20**, whereby it penetrates partially into the section of the fluid transport path **11** (sample layer **16*a***) situated beneath it.

For the evaluation the test kit is held with the first cover part **17** at the top, so that an eluant (preferably an aqueous solution containing auxiliary reagents, for example wetting agent and buffer) can be introduced through the recess **19** onto the suction layer **16*b*** situated beneath it. The eluant, which is applied in an amount sufficient to fill the whole capillary-active fluid transport path **11**, is transported by capillary forces along the delay section **13** to the sample application zone **14** (sample layer **16*a***). The flow rate is in so doing slowed down in such a way that it is reduced considerably in the region of the sample layer **16*a***. A substantially improved elution in the sample application zone **14** is thereby achieved.

In the case of the embodiment shown in FIG. **1** the testing of the analyte takes place by means of the eluate reception layer **16*c***. The latter can be separated and analysed by means of a reagent set forming part of the test kit. Alternatively the eluate reception layer **16*c*** itself is equipped with the reagents required for producing a test signal specific for the analysis, which is evaluated in a known manner (in the case of a colour change usually by reflection photometry).

FIGS. 2 to 6 show a preferred embodiment which is distinguished both by its operation (accurate and reproducible analysis results) and by simple and therefore inexpensive manufacture.

The test kit includes a sample collecting unit **30** and test carriers **31** which are adapted specifically to the latter (FIG. 3) and are advisably formed similarly to a conventional test strip. They contain in one or more superimposed or adjacent test layers a reagent system for determining a particular analyte. In the figure only one test layer **32** is indicated.

Within a test kit according to the invention use is preferably made of several types of test carriers **31***a*, **31***b*, which serve for the determination of different analytes (parameters) and therefore make a profile analysis of the stool possible in a very simple manner. In this way a far more exact diagnostic statement can be made on the existence of a particular condition. It is particularly advantageous that the profile is selective, i.e. that only those parameters have to be determined by the doctor which are required in connection with the suspected diagnosis. Moreover, the analysis is for practical purposes just as easy to carry out as the stool blood tests current today, which are carried out in very large numbers as part of health screening.

The sample collecting unit **30** consists of only two parts, namely a blank designated as a folding part **34** and consisting of water-proof-coated, thin (not more than approx. 500 g/m^2) cardboard and a insert part **16'** consisting of a porous, capillary layer material (FIG. **4** and FIG. **6**). In principle, both natural materials and (preferably) hydrophillic plastics are suitable as capillary layer material, which are processed to produce the capillary-forming structures as papers, mats, fabrics or correspondingly porous and structured films. A mat of hydrophillic plastics fibres is particularly preferred.

The folding part **34** possesses three main folding lines **35**, **36** and **37**, by means of which four sections **1** to **4** are divided off which are folded for the manufacture of the sample collecting unit **30** in the manner shown in FIG. **5**.

The section **1** of the folding part **34** serves mainly as a bearing part **40** for the insert part **16'**. The insert part **16'** is fixed to the bearing part **40** in such a way that its capillary-active properties are not impaired by the fixing. Point- or strip-wise adhesion at the edge of the insert part **16'**, for example, is suitable.

The sections **2** and **3** form the second cover part **18'** with the recess **20'**, which frames the sample field **23'**, and the first cover part **17'** with the recess **19'**, which makes the eluant application field **24'** accessible.

This layered structure can be seen most clearly in FIG. **6**, in which the thickness of the layers shown is exaggerated. The actual thickness of the layer material of which the folding part **34** is manufactured is preferably less than 0.5 mm, so that a packet with a thickness of less than about 2 mm can be folded out of it.

A preferred configuration of the fluid transport path **11'**, in particular of the delay section **13'**, can be seen in FIG. **4**. The eluate application field **24'** in the suction layer **16***b'* is relatively extended. From here the fluid transport path **11'** leads, via a sub-section **26** divided into two, to the sample layer **16***a'* with the sample field **23'** (indicated by dots in FIG. **4**) and from there to an eluate reception layer **16***c'*. It is characteristic of the preferred embodiment shown that the width of the sub-section **26** (sum of both branches) is less than the width of the fluid transport cross-section in the region of the sample field **23'**. It is in addition very long (approx. 50% of the overall fluid transport path). In order nevertheless to make a manageable overall size of the sample collecting unit **30** possible, the fluid transport path **11'** exhibits windings, at which the fluid transport direction undergoes a plurality of changes.

The eluant application field **24'** is accessible from the doctor's side (FIG. **3**) of the sample collecting unit through the recess **19'** in the first cover part **17'** and a further recess **41'** in the bearing part **40'**. The recess **19'** is sealable with a flap **45** swivellable about a broken line **44**.

The sample field **23'** is accessible from the patient's side (FIG. **2**) through the recess **20'** in the second cover part **18'**. It is sealable with a flap **46** which is swivellable about a broken line **47**. It is closed at its front edge by means of a tear line **48** when the sample collecting unit **30** is not is use.

The eluate reception zone **15'** is provided with eluate transfer means designated overall as **50**. The eluate transfer means **50** and the test carriers **31** are adapted to one another so that eluate present in the eluate reception region **15'** can be transferred onto the at least one test layer **32** of the test carrier.

The eluate transfer means **50** comprise in the case shown the eluate reception layer **16***c'*, a distance recess **51** in the second cover layer **18'** and a test carrier opening **53** sealed with a flap **52** when the sample collecting unit **30** is in its initial state. As can be seen from FIG. **6**, the dimensions are coordinated with one another so that a test carrier engaging with the distance recess **51** through the opening **53** is with its test field **32** in fluid contact with the eluate reception layer **16***c'*.

In the preferred case shown the eluate reception layer **16***c'* is widened so that by means of three eluate transfer devices **50**, **54** and **55** fluid contact with three test carriers introduced into the openings **53**, **56** and **57** and differing with respect to the analyte for which they are specific can be produced. In this way the determination of an analysis profile from several parameters is possible. A particularly preferred combination includes the following determinations:

1. Conventional test for concealed blood, in particular by means of a haemoglobin-peroxidase test.
2. Immunological determination of HSA as per EP-A-291 843.
3. Determination of leucocytes by an enzymatic test of the leucocyte elastase.

The determination of leucocytes by testing for their elastase activity has been developed for the analysis of urine. The possibility of testing for leucocytes in stool has also already been indicated (EP-A-0 012 957). Although the diagnostic relevance of this test, which in particular can provide useful pointers as to the presence of inflammatory disorders, is undisputed, there is to date no easily manageable and reliable methodology enabling this test to be carried out in practice. A test kit for such a determination is provided by the present invention. It has been shown, surprisingly, that particularly good results with regard to sensitivity on the one hand and selectivity on the other (avoidance of false negative and false positive findings) are achieved if, on the basis of this invention, a relatively high concentration of the analyte in the eluate is ensured and on the other a relatively insensitive leucocyte test is worked with. It has been found that a particularly suitable test procedure is the procedure described in EP-B-0 012 957 including the improvements described in EP-B-0 014

929. The detection limit of the leucocytes is preferably fixed at more than 500 leucocytes/μl, particularly preferably more than a thousand leucocytes/μl.

The carrying out of the analysis is very simple:

The patient introduces the stool sample with a spatula through the recess **20'** onto the sample area **23'** and closes the flap **46**. The sample collecting unit **30** is so constructed that a small quantity of air reaches the sample, so that the latter dries out during storage.

For the testing of the sample the test carrier openings **53, 56** and **57** and the flap **45** sealing the eluant application field **24'** are opened. Depending on the desired analysis profile, one or more test carriers are introduced into the eluate transfer devices **50, 54** and **55**. A measured amount of eluant is metered onto the eluant application field **24'**. The test carrier **31** is removed at a predetermined time and the test signal is evaluated visually or with an apparatus.

While there has been described what is at present considered to be the preferred embodiment of this invention, it will be obvious to those skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is, therefore, aimed to cover all such changes and modifications as fall within the true spirit and scope of the invention.

What is claimed is:

1. A test kit for determining an analyte in a pasty spreadable sample containing solid constituents, comprising:

an eluant feed zone;

an eluate reception zone for receiving an eluate liquid;

a fluid transport path means for transporting a liquid by capillarity in a flow direction which leads from said eluant feed zone via a sample application zone to said eluate reception zone, said sample application zone having a porous sample layer comprising a sample field for the application of the pasty solid containing sample to partially penetrate into the porous sample layer;

a delay section in the fluid transport path between the eluant feed zone and the sample field, said delay section comprising delay means for controlled reducing of the fluid transport rate in the sample layer and having a fluid transport rate which is smaller by at least a factor of two than the fluid transport rate of the sample layer in the sample field, each of said fluid transport rates being measured in isolation;

a covering part which covers at least said sample application zone and includes means for providing access to said sample field for the pasty spreadable solid containing sample to be applied to and cover said sample field; and

analysis means containing reagents which react with the analyte and comprise a component producing a test signal.

2. The test kit of claim **1** wherein the sample field has a longitudinal dimension in the flow direction and the delay section is substantially longer than said sample field in that direction.

3. The test kit of claim **1** wherein the sample layer has a fluid transport cross-section and the delay section comprises a flat layer material for transporting a liquid by capillarity, said delay section having a fluid transport cross-section and comprises a narrowing in which the fluid transport cross-section of the delay section is smaller than the fluid transport cross-section of the sample layer.

4. The test kit of claim **3** wherein the narrowing is a constriction at which the layer material is compressed perpendicular to its surface dimension.

5. The test kit of claim **3** wherein the sample field has a sample layer and the narrowing is a sub-section of reduced width in relation to the sample layer width.

6. The test kit of claim **1**, further comprising a sample collecting unit including the fluid transport path and which includes eluate transfer means in the eluate reception zone and in which the analysis means includes a test carrier with at least one test layer, and in which the test carrier and the sample collecting unit are adapted so that eluate present in the eluate reception zone is transferred by means of the eluate transfer means to said least one test layer of the test carrier.

7. The test kit of claim **6** wherein the eluate reception zone comprises a plurality of eluate transfer means each of which is in fluid communication with the fluid transport path.

8. The test kit of claim **1** wherein at least a part of the fluid transport path comprises an insert part including a porous capillary layer material.

9. The test kit of claim **8** wherein the covering part comprises a first cover part and a second cover part, said first and second cover parts encasing the insert part therebetween, and the cover parts having in each of the eluant application zone and the sample application zone means through which the fluid transport path of the insert part is accessible.

10. The test kit of claim **9** further comprising a bearing part of water-proof layer material to which the insert part including porous layer material is fixed, the water-proof layer material being encased between the cover parts together with the insert part.

11. The test kit of claim **1** wherein the analysis means include a reagent system for determining an elastase.

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